THE ROLE OF PITUITARY BETA-ENDORPHIN IN THE ATTENUATION OF NOCICEPTION

1986

HARGREAVES

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
AUG 1986	**N/A**	**-**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
The Role of Pituitary Beta-Endorphin in the Attenuation of Nociception	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Uniformed Services University Of The Health Sciences Bethesda, MD 20814**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**SAR**	**252**	

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BETHESDA, MARYLAND 20814-4799

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Title of Thesis: The Role of Pituitary Beta-Endorphin in the Attenuation of Nociception

Name of Candidate: Kenneth Hargreaves
Doctor of Philosophy Degree
August 28, 1986

Thesis and Abstract Approved:

Committee Chairperson	8/28/86 Date
Committee Member	8/28/86 Date
Committee Member	8/28/86 Date
Committee Member	8/28/86 Date
Committee Member	8-28-86 Date

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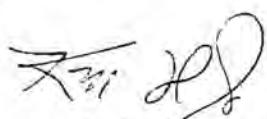

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This dissertation and equally important, the years invested in training at Berkeley, Davis, Georgetown and USUHS, are dedicated to Holly, for her continuous love, support and patience.

ACKNOWLEDGEMENTS

I am grateful for the friendship and seemingly endless patience of Doctors Gregory Mueller and Raymond Dionne, who guided me through the basic and clinical studies. I owe a lot to the thoughtful comments and encouragement of Doctors Brian Cox, Modi Pamnani and James Terris. Doctors Loring Chapman, Arnold Lieman, Howard Bern and JoAnn Nuite were essential in my earlier training and guidance. I also wish to thank the many patients at the NIDR who voluntarily gave of themselves to provide the information that we learned from the clinical studies. Finally, mange tusen tak to Red, Erna, Noël, Gudrun, Chris, Ted and Leslie for their years of support and advice.

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ABBREVIATIONS

Abbreviation	Name
ACTH	Adrenocorticotropin, corticotropin
AVP	Arginine vasopressin, antidiuretic hormone
b.i.d.	(bis in die) twice a day
BBB	Blood brain barrier
B-END	Beta-endorphin
B-LPH	Beta-lipotropin
C	Centrigrade
CNS	Central nervous system
CRF	Corticotropin releasing factor
CRH	Corticotropin releasing hormone
CSF	Cerebrospinal fluid
DEX	Dexamethasone
ED50	Effective dose for 50% of the population
Fig	Figure
fm/ml	Femtomoles per milliliter
FSH	Follicle stimulating hormone
GNRH	Gonadotropin releasing hormone
GH	Growth hormone
HTM	High threshold mechanoreceptor
HYPOX	Hypophysectomized
iB-END	Immunoreactive beta endorphin
i-cortisol	Immunoreactive cortisol
i.c.v.	Intracerebroventricular
i.p.	Intraperitoneal
i.v.	Intravenous
Leu-ENK	Leucine enkephalin
LH	Luteinizing hormone
Met-ENK	Methionine enkephalin
mg	Miligram
mg/kg	Miligram of drug per kilogram body weight
min	Minute
NAL	Naltrexone
nmol/kg	Nanomoles of drug per kilogram body weight
NS	Non-significant
POMC	Proopiomelanocortin
pg/ml	Picrograms/milliliter
PRL	Prolactin
Q-NAL	Quaternary analog of naltrexone, naltrexone methylbromide, MRZ 2663
RIA	Radioimmunoassay
sc	Subcutaneous
sec	Second
SHAM	Sham surgery (gland is left intact)

Abbreviation	Name
SIA	Stress-induced analgesia, stress-induced antinociception
TRH	Thyrotropin releasing hormone
ug/dl	Micrograms per deciliter
ul	Microliter
umol/kg	Micromoles of drug per kilogram of body weight

CHAPTER 1

INTRODUCTION

1-1. Overview of Project

This research project examined the relationship between pituitary beta-endorphin (B-END) levels and pain* in both humans and animals. The clinical trials examined this relationship through pharmacological interventions, both stimulatory and inhibitory, on release of pituitary B-END in patients prior to the onset of acute post-operative pain. Experiments using laboratory animals further investigated this relationship through interventions of both endocrine and opiate activity.

The clinical experiments:

1. Established that the oral surgery model is appropriate for investigating the release of pituitary B-END immunoreactivity in humans undergoing surgical stress and post-operative pain.

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* When discussing animal studies, the terms "nociception" and "antinociception" are used in deference to the terms "pain" and "analgesia"; since, in animal studies, the dependent measure is a change in behavior, which may result from drugs altering motor responsiveness in addition to those producing analgesia. The terms "pain" and "analgesia" will be employed in describing clinical studies.

2. Examined the relationship between release of pituitary B-END with changes in post-operative pain and perceived anxiety following intravenous administration of the hypothalamic hormone, corticotropin releasing hormone (CRH), an opiate agonist, fentanyl, an opiate antagonist, naloxone, a synthetic glucocorticoid, dexamethasone and a benzodiazepine, diazepam.

The animal experiments:

1. Determined dose related effects of intravenous administration of CRH on circulating levels of immunoreactive beta-endorphin (iB-END) and antinociception in laboratory rats.
2. Determined the effects of pituitary interventions (dexamethasone suppression of the pituitary-adrenal axis and hypophysectomy) on CRH-induced changes in behavioral nociception.
3. Determined the effects of endorphin interventions (passive immunization, naltrexone and naltrexone methylbromide - a quaternary analog of naloxone) on CRH-induced changes in behavioral nociception.

These parallel studies, conducted in both humans and animals, determined the relative contribution of pituitary B-END peptides to the modulation of nociception in both a clinical and an experimental model of pain.

1-2. Endogenous Opioid Peptides

1-2.a. Overview

Although exogenous opiates have been in use since the time of the Sumerians (Jaffe and Martin 1985), the search for endogenous opioid substances was prompted by two relatively recent discoveries. The first discovery was the initial demonstration of stereospecific opiate receptor binding sites in the central nervous system (CNS) (Goldstein, et al. 1971), which was subsequently confirmed and extended (Pert and Snyder 1973, Simon, et al. 1973, Terenius 1973). This finding provided a rationale for research attempting to isolate endogenous opioid substances, since the argument was raised that it was unlikely that CNS opiate receptors would exist solely for exogenous alkaloids from the poppy plant. The discovery of opiate receptors constituted a substantial incentive for isolating endogenous opioid substances. The second key discovery was the development of selective bioassays for the detection of endogenous opioids in tissue extracts. Hughes and Kosterlitz successfully employed the guinea pig ileum and mouse vas deferens bioassays to detect the presence of endogenous opioid factors, which led to their discovery of the enkephalins in 1975 (Hughes 1975, Hughes, et al. 1975). Thus, the fundamental observation of opiate receptor binding sites and utilization of sensitive

bioassays underlie the discovery of the endogenous opioid peptides.

Three families of opioid peptides are known to exist, derived from proenkephalin, prodynorphin and proopiomelanocortin, which are related by containing a common tetrapeptide sequence of amino acids (TYR-GLY-GLY-PHE). The amino acid sequence for all three precursors has been confirmed using molecular biology techniques (Noda, et al. 1982, Gubler, et al. 1981, Kakidana, et al. 1982, Nakanishi, et al. 1979). The nomenclature employed herein follows that reviewed by Cox (1982) and Kitchen (1984).

1-2.b. Proenkephalin Related Peptides

The proenkephalin amino acid sequence contains 4 repeats of the Met-enkephalin (Met-ENK) structure, one Leu-enkephalin (Leu-ENK) and one Met-ENK-Arg-Gly-Leu peptide structure (Gubler, et al. 1981). Proenkephalin is located in peripheral tissues such as the adrenal medulla, sympathetic ganglia, peripheral nerves and the gastrointestinal tract (Hughes 1975, Schultzberg, et al. 1979, Smith, et al. 1976). CNS locations for proenkephalin perikarya include striatum, midbrain, pons, medulla and spinal cord (Hughes 1975). The terminals appear to be well distributed throughout the CNS (Akil, et al. 1984).

1-2.c. Prodynorphin Related Peptides

Within the amino acid sequence of prodynorphin lies three Leu-ENK peptide cores, which appear to reside as part of alpha and beta neo-dynorphin, dynorphin A and dynorphin B. Alternative forms of post-translational processing appear possible and, therefore, specific forms of peptides derived from prodynorphin may be tissue-specific. Prodynorphin has been located in peripheral tissues such as the duodenum, posterior pituitary (perikarya located in the magnocellular region of the supraoptic nucleus of the hypothalmus), and in the CNS in areas such as the caudate, putamen, spinal cord dorsal horn and in several medullary nuclei (Goldstein, et al. 1979, Goldstein, et al. 1981, Tachibana, et al. 1982, Watson, et al. 1982).

1-2.d. Proopiomelanocortin Related Peptides

Unlike proenkephalin and prodynorphin, proopiomelanocortin (POMC) gives rise to only one opioid peptide product. Although B-END_{1-31} contains the M-ENK sequence on its N-terminal, B-END_{1-31} is, in fact, the final opioid cleavage product of POMC and Met-ENK is not released as such from POMC processing. POMC peptides are most highly concentrated in the lobes of the pituitary which contain approximately three orders of magnitude more iB-END than the CNS. Additionally, the placenta, reproductive tract, lymphocytes, gastrointestinal tract and

lungs are reported to contain small amounts of POMC peptides (reviewed by Krieger 1983). In the CNS, POMC perikarya appear to cluster in two areas, the arcuate nucleus and the nucleus tractus solitarius (Bloch, et al. 1978, Bloom, et al. 1978, Schwartzberg and Nakane 1981). POMC fibers projecting from the arcuate nucleus terminate in several limbic and brain stem regions including periaquaductal grey, rostral medullary nuclei, trigeminal nuclei and parabracheal nuclei. The distribution of POMC fibers from the nucleus tractus solitarius is not as well characterized.

Post-translational processing of POMC is tissue specific and POMC derived peptides include adrenocorticotropin (ACTH), melanocyte stimulating hormone (MSH), corticotropin-like intermediate peptide (CLIP), beta lipotropin (B-LPH), and acetylated or non-acetylated B-END 1-31, 1-27, 1-26. Three patterns of processing have been reported. In the first pattern, observed in the pars distalis of the pituitary, POMC is cleaved to ACTH and B-LPH, with less $B\text{-}END_{1-31}$. In the second pattern, found in the pars intermedia of the pituitary, post-translational processing of POMC gives rise to alpha MSH, CLIP, and primarily shorter and acetylated forms of B-END. The third pattern of POMC processing occurs in the hypothalmus and reproductive tract, with predominant formation of alpha MSH and shorter forms of B-END. It is unclear to what extent CNS POMC gives rise to acetylated B-END, with several labs

reporting different findings (reviewed by Krieger 1983). This point may be clarified by the work of Millington, et al. (1984) who demonstrated that the relative ratio of POMC derived peptides varied in different areas in the CNS.

1-3. Proposed Physiological Functions of B-END

1-3.a. Overview

The successful recovery from stress requires a series of coordinated physiological responses. Initial observations by Selye (1936) led to his proposal that a major physiologic response to stress is activation of the pituitary-adrenal axis. Evidence advanced by Selye and others led to the conclusion that stress evokes pituitary secretion of ACTH, which in turn stimulates the synthesis and release of adrenal glucocorticoids. The vital importance of this endocrine axis in mediating adaptive responses to stress is emphasized by the fact that patients with a non-functional pituitary-adrenal axis (e.g. Addison's Disease) do not survive physical stress well (Baxter and Tyrrell, 1981). Stimulation of the pituitary corticotroph releases ACTH, B-END and B-LPH in response to a variety of stressors in both animals and in humans (Guillemin, et al. 1977, Mueller 1981, Dubois, et al. 1981). While the physiological functions of ACTH are well

recognized, the contributions of B-END and B-LPH, if any, in mediating adaptive responses to stress are unknown.

This section reviews the proposed physiological functions of B-END. Two initial points should be noted. First, most of the literature reports data following administration of B-END in pharmacological doses, which far exceed physiological levels. Davenport (1982) summarizes this general issue of dosage in his monograph on gastrointestinal physiology:

> The chemical and pharmacological problem is to discover all of the properties and functions of a messenger. The physiological problem is to determine which of these is important in governing the behavior...

Thus, observed effects may indeed be due to B-END, but may be unrelated to its actual physiological functions.

The second point to note is that efforts to understand the physiological functions of B-END are complicated by both the biochemical heterogeneity of endogenous opioid peptides and the anatomical diversity of POMC-derived peptides. Problems due to biochemical heterogeneity further relate to the multiple classes and locations of opiate receptors, suggesting that pharmacological administration of B-END may activate opiate receptors normally inaccessable (either due to low affinity or to location) to pituitary B-END. Due to the anatomical diversity of POMC gene expression, the physiological

correlate of observed effects following pharmacological administration of B-END may be due either to local synthesis of B-END (e.g. lymphocytes), or to pituitary secretion of B-END, or to some combination of local and pituitary B-END. For example, B-END stimulates several components of the immune system. However, this may be unrelated to pituitary B-END, since immunoreactive POMC peptides are reportedly synthesized by leukocytes (see below). Together, these points suggest that many of the studies reporting effects of B-END contain data unrelated to the physiological actions of pituitary B-END. Thus, the following review indicates only possible functions of pituitary B-END.

1-3.b. Pituitary Hormones

Studies evaluating secretory changes in pituitary function following administration of opiates only demonstrate the capacity to respond - none have adequately addressed the origin of the actual physiologic signal, either a paracrine pituitary opioid effect, or via an opioid (either of pituitary or of hypothalamic origin) alteration in the secretion of a hypothalamic releasing factor, or possibly both. The hypothesis that pituitary B-END modulates the release of other pituitary hormones is complicated by observations of Wardlaw, et al. (1980) that the arcuate nucleus appears capable of secreting B-END into

portal vessels, where it could then gain access to pituicytes. It appears possible that hypothalamic and pituitary pools of B-END may independently modulate pituitary activity.

Several lines of evidence have implicated opioid modulation of prolactin (PRL) secretion. Administration of B-END, as well as opiates, stimulated PRL secretion in both humans and rats (Rivier, et al. 1977, Kato, et al. 1978, Reid and Yen 1981, Foley, et al. 1979). Although naloxone administration did not alter basal levels of PRL (Morley, et al. 1980, Janowsky, et al. 1978, Martin, et al. 1979), naloxone (Van Vugt, et al. 1978, Grandison and Guidotti 1977) did suppress stress-induced secretion of PRL.

Studies employing pituitary cultures have demonstrated a direct effect of B-END in stimulating PRL release and in reversing dopamine suppression of PRL secretion (Cheung 1984, Enjalbert, et al. 1979). However, this potential paracrine effect of pituitary B-END may not play a major physiological role in PRL regulation. This conclusion is based on the findings that intracerebroventricular (i.c.v.) passive immunization with anti-endorphin antiserum significantly suppressed baseline and stress-induced increases in PRL (Ragavan and Frantz 1981a) whereas intravenous (i.v.) passive immunization was without effect (Ragavan and Frantz 1981b). Ragavan and Frantz interpret these data to indicate that central pools

of B-END and not pituitary B-END play a predominant role in modulating PRL release.

Since impotence and reproductive dysfunctions are classic sequelae of opiate addiction, it is not surprising that research has been conducted to elucidate the underlying physiological mechanisms. Numerous studies have demonstrated that administration of opiates and opioid peptides including B-END, resulted in a prompt suppression of luteinizing hormone (LH) levels (Reid and Yen 1981, Bruni, et al. 1977, Foley, et al. 1979, Zimmerman and Pang 1976). Opiates have a variable effect on secretion of follicle stimulating hormone (FSH), with most studies (Meites, et al. 1979, Cicero, et al. 1977, Snowden, et al. 1984), but not all (Zimmerman and Pang 1976) failing to observe changes in FSH levels. Opiates may have a tonic inhibitory effect on gonadotropin secretion since administration of naloxone consistantly increased LH and FSH levels (Bruni, et al. 1977, Meites, et al. 1979, Morley, et al. 1980, Wiesner, et al. 1985). However, it is unlikely that this action is mediated by a local effect of pituitary B-END, since opiates have no effect on gonadotropin secretion in vitro at concentrations up to 10 umolar (Meites, et al. 1979, Wiesner, et al. 1984). Instead, it is likely that hypothalamic B-END plays a role in modulating the preovulatory surge of LH (Sarker and Yen 1985, Wiesner, et al. 1984).

Pharmacological administration of opioids also has been demonstrated to stimulate growth hormone (GH) release. B-END, administered either centrally or peripherally, stimulated secretion of GH (Katakami, et al. 1981, Morley 1981, Rivier, et al. 1977, Kato, et al. 1978, Cusan, et al. 1977, Dupont, et al. 1977). However, it does not appear that endogenous opioids play a major role in maintaining basal levels of GH, since naloxone administration generally (Morley, et al. 1979, Martin, et al. 1979) but not always (Bruni, et al. 1977) had little to no effect on GH levels. In addition, passive immunization with anti-B-END antisera (i.v.) did not alter GH levels (Tannebaum, et al. 1979). Finally, administration of CRH, which stimulates release of pituitary B-END, has not been observed to stimulate GH release in humans or rats (Copinschi, et al. 1983, Hermus, et al. 1984, Orth, et al. 1983, Rivier and Vale 1984a), although it reportedly stimulated GH release in stalk sectioned macaques (Schulte, et al. 1982). While further work is required, it appears that pituitary B-END probably plays a minor role, if any, in regulating GH release.

The effect of opioids on secretion of thyroid stimulating hormone (TSH) is dependent on their site of administration. Central administration of B-END and opiates resulted in a suppression of TSH levels (Lomax, et al. 1970, Judd and Hedge 1982). Conversely, microinjections of B-END into the pituitary (Judd and Hedge 1982) and administration of B-END to pituitary cultures

(Judd and Hedge 1982, 1983) both resulted in a stimulation of TSH release. In the pituitary superfusate model, B-END increased the perfusate levels of TSH by 50% within 1 minute of opioid administration (Judd and Hedge 1983). It is likely that pituitary B-END may exert local effects on modulating thyrotroph secretion of TSH under conditions of B-END secretion.

1-3.c. Other Endocrine Glands

Peripheral endocrine glands may also serve as target sites for circulating B-END. Perfusion of B-END, or morphine, into the canine pancreas resulted in secretion of insulin and glucagon with decreased levels of somatostatin in venous blood (Ipp, et al. 1978). Subsequent studies have demonstrated that administration of opiates both in vivo and in vitro altered secretory activity of the endocrine pancreas, although a biphasic dose response curve may be observed (Hart and Cowie 1978, Pyke 1979, Kanter, et al. 1979, Reid and Yen 1981, Feldman, et al. 1983).

Isolated adrenal cortical fasiculata cells responded to B-END administration with increased synthesis of corticosterone (Shanker and Sharma 1979). B-END induced a six fold increase in corticosterone levels over control; however, this effect may not be of physiological significance, since B-END was only 0.1% as potent as ACTH

and the ceiling for B-END was approximately 50% that of ACTH (Shanker and Sharma 1979).

Numerous studies have demonstrated that aldosterone levels are increased following administration of POMC-derived peptides (Matsuoka, et al. 1981, 1984, Washburn, et al. 1982, Vinson 1980, Vinson, et al. 1981, Nicholls 1975). Moreover, administration of CRH to human subjects resulted in increased circulating levels of aldosterone (Nakahara, et al. 1983, Conaglen, et al. 1984, Hermus, et al. 1984). However, it is unclear to what extent B-END, as compared to other POMC-derived peptides (e.g., B-LPH, ACTH and B-MSH), underlies the observed changes in aldosterone. B-END has been observed to stimulate aldosterone secretion under some (Gullner and Gill 1983, Rabinowe, et al. 1985), but not all experimental conditions (Kem, et al. 1985, Szalag and Stack 1981, Matsuoka, et al. 1981). Continued research is necessary to determine the relative contribution of B-END in modulating the secretion of aldosterone.

1-3.d. Immune System

Endogenous opioid peptides, including B-END, have been demonstrated to modulate several aspects of the immune system. However, this effect may be due to local B-END via leukocyte synthesis (Blalock and Smith 1980, Smith and Blalock 1981, Lolait, et al. 1984), or to pituitary B-END, or to both local and pituitary B-END. Early reports

demonstrated that lymphocytes contain opiate binding sites (demonstrated by naloxone blockade) as well as endorphin binding sites (binding apparently near the C-terminal of B-END) (Hazum, et al. 1979, Mehrishi and Mills 1983). Subsequent studies demonstrated that B-END enhanced rat spleen T lymphocyte activity (Gilman, et al. 1982) and suppressed human T lymphocyte activity (McCain, et al. 1982). More recent studies have failed to demonstrate opiate induced alteration of human lymphocyte proliferative responses to a variety of in vitro challenges (Wybran 1985).

A clearer picture emerges from studies on endorphin activation of chemotaxis. B-END exerted a positive chemotactic effect on isolated human monocytes in concentrations as low as 1 pM (Van Epps, et al. 1983, Van Epps and Saland 1984, Simpkins, et al. 1984). In addition, i.c.v. injection of B-END into rats resulted in an accumulation of macrophage-like cells within 1 hour (Van Epps, et al. 1983, Van Epps and Saland 1984, Saland, et al. 1983). The relevance of the observed chemotaxis to physiological levels of B-END must be questioned, however, since the pituitary maintains a high vascular concentration gradient of B-END, and yet does not apparently contain inordinate concentrations of leukocytes.

B-END has also been demonstrated to stimulate cytotoxicity, the ability of leukocytes to kill non-recognized cells. Froelich and Bankhurst (1984)

demonstrated that B-END potentiated the ability of monocytes to destroy tumor cells (a model of natural killer activity), but not to alter antibody dependent cytotoxicity. This observation has been confirmed in other reports (Mathews, et al. 1983, Kay, et al. 1984) and extended by observing similar effects for gamma endorphin and B-LPH. B-END also binds to various forms of human complement (Schweigerer, et al. 1982a,b, Schweigerer 1983, Schweigerer, et al. 1983), suggesting its involvement in modulating processes which lead to cell lysis.

Together, these studies indicate that B-END may activate and modify the ongoing activity of the immune system in response to an environmental challenge. Thus, the pituitary-adrenal axis activity may possibly alter immune function by both circulating B-END as well as glucocorticoids (Munck, et al. 1984, Flower, et al. 1986).

1-3.e. Cardiovascular System

Several lines of evidence implicate opioid modulation of cardiovascular activity, although the observations are variable and appear to be dependent on the experimental paradigm (i.e., awake vs anesthetized animals, species, dose, route). For example, opiate agonists tend to increase blood pressure when administered to awake animals and tend to decrease blood pressure when administered to anesthetized animals (reviewed by Olson, et

al. 1984, 1985). Opiate binding sites have been detected in isolated rat hearts (Krumins, et al. 1985). These binding sites may represent physiological receptors, since the number of available binding sites is significantly diminished following severe hemorrhage, perhaps indicating increased occupancy by endogenous opioid peptides (Krumins, et al. 1985). Moreover, B-END decreased developed pressure in the in vitro rat heart preparation (Lee, et al. 1984).

In a series of studies, Kiang and Wei (1984, 1985) have demonstrated that pituitary B-END produces a bradycardia through activation of opiate receptors. Administration of CRH (6 nmol/kg) produced a bradycardia in their model of urethane anesthetized rats. This CRH-induced bradycardia appears to be due to release of pituitary B-END, since it is blocked by naloxone, hypophysectomy and dexamethasone pre-treatment (Kiang and Wei 1985). Interestingly, the CRH bradycardia was blocked by naloxone (4 mg/kg, i.v.), but not by the quaternary analog of naloxone (16 mg/kg, i.v.) which is excluded by the blood-brain-barrier (BBB). Kiang and Wei interpreted these findings to indicate that CRH induced bradycardia is due to pituitary secretion of opioid POMC peptides, which probably act in the CNS to produce the observed effect.

In addition to its effects on cardiac function, peripheral B-END may also alter vascular tone. For example, B-END caused arteriolar vasodilation in the hamster (Wong, et al. 1981, Koo and Wong 1982). This

effect may be similar to the generally observed actions of opiates on inhibiting smooth muscle contractions following nerve stimulation in other vascular preparations (Illes, et al. 1983, Yamamoto, et al. 1984, Ruth, et al. 1984).

Thus, B-END appears to possess cardiomodulatory properties which, as suggested by the work of Kiang and Wei (1984, 1985), may be a physiological function of pituitary B-END.

1-3.f. Gastrointestinal System

Considering the pivotal role that the guinea pig ileum bioassay had on the discovery of the endogenous opioids, it is not surprising that B-END (as well as opiates) has been implicated in the modulation of gastrointestinal activity. Several studies have demonstrated that B-END inhibits intestinal contractions (Galligan, et al. 1984, Burks, et al. 1982) and opiates in general have been shown to delay intestinal transit time (Parolaro, et al. 1983). These observations are thought to form the basis for the antidiarrheal action of opiates (Powell 1981). The effect of B-END on gastric acid secretion is less clear. B-END has been reported to inhibit gastric acid secretion in some (Morley 1981, Morley, et al. 1982b), but not all (Feldman and Li 1982) studies. B-END has also been demonstrated to inhibit the

formation of gastric ulcers following stress in the rat (Hernandez, et al. 1983).

1-3.g. Other Potential Targets

B-END has also been implicated in modulating the activity of other peripheral target sites. A receptor for B-END has been recently discovered in rat liver, kidney, adrenal, spleen and testes (Dave, et al. 1985a). This receptor recognizes the mid-portion of B-END, since it binds to B-LPH and acetylated B-END_{1-27}, but does not recognize morphine, met-enkephalin or gamma endorphin (= B-END_{1-17}). Accordingly, it is referred to as a "non-opiate" endorphin receptor. Interestingly, occupation of this receptor at physiological levels of B-END resulted in a stimulation of adenylate cyclase activity (Dave, et al. 1985a). Thus, pituitary B-END may alter the functional activity of a variety of peripheral target sites.

1-3.h. Participation in Endogenous Pain Suppression Systems

Following the structural identification of endogenous peptides capable of binding to opiate receptors, intense efforts were made to determine their analgesic properties. These studies examined antinociception produced by B-END following either central or peripheral

administration into animals. It was quickly reported and corroborated that B-END administered i.c.v. to rats was, on a molar basis, 3 to 116 times more potent than morphine in producing antinociception using either the hot plate or tail flick models (Loh, et al. 1976, Bradbury, et al. 1977, Tseng, et al. 1976a, Nicolas, et al. 1984, Holaday, et al. 1977, Tseng, et al. 1977, Inturrisi, et al. 1980, Graf, et al. 1976, Nemeroff, et al. 1979, Tseng, et al. 1979). Similarly, clinical trials have demonstrated that intraventricular or intrathecal administration of B-END produced profound relief from chronic pain (Oyama, et al. 1980, Hosobuchi and Li 1978, Foley, et al. 1979, Wen, et al. 1985) although these studies were not generally conducted under double-blind, randomized, placebo controlled conditions. Thus, centrally administered B-END possesses potent antinociceptive properties in animal and probably human models of pain.

Other studies have attempted to determine whether B-END possesses antinociceptive properties by intravenous administration. Interestingly, when peripherally administered in mice, rats or cats, B-END was approximately 3.2 to 100 times more potent than morphine on a molar basis (Holaday, et al. 1977, Tseng, et al. 1976a, Holaday, et al. 1979, Feldburg and Smyth 1976). The antinociception began as early as 5 minutes following i.v. administration and persisted for about 45 minutes (Tseng, et al. 1976b). Tseng's data on relative potency comparisons between

intravenous B-END and morphine sulfate indicated that, when compared on a molar basis (i.e. umol/kg), B-END is 4.2 times more potent than morphine on the tail flick and 3.2 times more potent on the hot plate model. In addition, limited clinical studies have demonstrated relief from intractable pain following intravenous administration of B-END at some (Catlin, et al. 1978) but not all (Foley, et al. 1979) doses. Together, these limited data are consistent with the hypothesis that intravenously administered B-END produces an antinociception in animals and analgesia in humans.

However, the physiologic significance of these data is unclear. In general, peripheral administration studies do not adequately reproduce conditions following pituitary secretion of endogenous B-END. Several problems are inherent with this type of experimental design. The first problem in interpreting these studies is that blood levels of B-END following i.v. injection have been calculated to be in great excess of levels obtained following maximal pituitary secretion. Thus, antinociception could represent a pharmacologic effect and not be representative of physiologic actions of pituitary B-END.

Conversely, greater doses of B-END may be required since i.v. administration does not mimic pituitary secretory patterns. In other words, pituitary B-END may be released into both the systemic circulation and hypophyseal portal circulation, whereas i.v. injection is into systemic

vessels only. Secretion into the hypophyseal portal system would allow high concentrations of B-END to reach the CNS via a direct, retrograde flow. The portal system could play a significant role for pituitary B-END transport. This is supported by observations by Oliver, et al. (1977), who demonstrated that hypophyseal portal vessel concentrations of ACTH fell from 82 ± 17 ng/ml to 0.61 ± 0.07 ng/ml within one hour of hypophysectomy. Accordingly, high concentrations of pituitary B-END might enter the median eminence region via portal vessel circulation. Once in the median eminence area, B-END could diffuse to nearby regions including the medial preoptic area and anterior hypothalmus. These areas are exquisitely sensitive to B-End for producing antinociception (Tseng, et al. 1980). However, it is currently unknown whether the concentrations or diffusion patterns of pituitary B-End to these regions are sufficient to produce antinociception.

An additional problem in interpreting i.v. injection studies is that the experimental conditions may be insufficient to evoke concomitant physiologic changes which would normally accompany stress-induced secretion of pituitary B-END. Once released into the systemic circulation, B-END exhibits only moderate permeability across the blood-brain-barrier (BBB). Under conditions of stress, however, the permeability of the BBB markedly increases (Pechura, et al. 1982). Pechura, and colleagues conducted a series of studies in which rats underwent a

conditioned fear training paradigm, where a conditioned stimulus was paired with a noxious, unconditioned stimulus (electrical shock) (Pechura, et al. 1982, Hayes, et al. 1985). Following the training, the permeability of the BBB in response to "psychological stress" (the conditioned stimulus) was evaluated by intravenous injection of horseradish peroxidase (HRP), a 40,000 dalton protein. In control rats, the mean number of HRP extravasation sites across the BBB was 15.7 + 1.8; while trained animals given the conditioned stimulus had twice as many HRP penetration sites, 33.0 + 1.5 ($p < .05$). Thus, the BBB appears to "open up" during a stressful event; this effect was most dramatic in the caudate nucleus, hippocampus, somatosensory cortex, lateral septum and trigeminal complex. Accordingly, environmental stressors sufficient to stimulate release of pituitary B-END may also increase its permability across the BBB.

Taken together, the data indicate that exogenous B-END, administered either intraventriculary or intravenously, is, on a molar basis, more potent than morphine for antinociceptive and analgesic effects. However, data from intravenous administration studies cannot be extrapolated to support potential antinociceptive functions of pituitary B-END. Instead, antinociception following peripheral administration of B-END may be viewed as a necessary criteria which is insufficient by itself to

provide compelling evidence that pituitary B-END modulates behavioral responses to pain.

1-4. Anatomical Substrates for Pain Perception and Modulation

1-4.a. Overview

Pain can be defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (International Association for the Study of Pain, Subcommittee on Taxonomy, 1979). Thus, the perception of pain is cued in terms of tissue damage and is comprised of both sensory and affective components. This section reviews fundamental anatomical substrates underlying the production of cutaneous pain, its rostral transmission and the centrifugal control of pain perception. Since extensive reviews of pain production, its rostral transmission (Yaksh and Hammond 1982, Dubner and Bennett 1983, Willis 1985) and its modulation by endogenous analgesic systems (Watkins and Mayer 1982, Dubner and Bennett 1983, Terman, et al. 1984, Basbaum and Fields 1984) have been recently published, this section will selectively emphasize the major components of current models of pain and generally use these secondary sources as entries for the literature.

The purpose of this section is to illustrate potential sites at which opioid peptides, either released from neurons or derived from the circulation, may act to modulate the perception of pain. A second objective is to introduce the concept that pain models (i.e., hot plate and clinical post-operative pain) are not all alike and differ considerably in their bioassay sensitivity and specificity. One example of a lack of bioassay sensitivity is the limited ability of the mouse hot plate model to detect antinociceptive effects of non-steroidal anti-inflammatory drugs, a class of drugs which are exceedingly potent analgesics in clinical models of post-operative pain (Taber 1974, Wood 1984, Chapman, et al. 1985, Dionne, et al. 1983, Troullos, et al. 1986). A second, classic example of differences in bioassay sensitivity, is the surprising clinical analgesic activity of nalorphine, which has no antinociceptive activity in animal studies (Lasagna and Beecher 1954). This finding, made using Beecher's clinical methodology (i.e. post-operative pain model), has been recently described by Lasagna (1984):

> One of the interesting contributions of the new methodology was the accidental finding by Lasagna and Beecher that nalorphine was an analgesic. Animal experiments gave no hint of this possibility. The realization came from clinical trials...

Thus, determination of analgesic actions of new drugs, or of physiological substances (e.g. B-END), should be made

using different bioassays for analgesics. Accordingly, lack of antinociceptive activity in any one bioassay should not be interpreted to indicate lack of analgesic activity in all bioassays. An analogy of this concept is the field of hypertension research, where there are several models of hypertension (e.g., clinical model, Goldblatt preparation, and spontaneous hypertensive rats) which differ in their responsiveness to various therapeutic agents.

1-4.b. Peripheral Nociceptors

Primary afferent neurons transmitting nociceptive information have been classified on the basis of their conduction velocities and response characteristics (Dubner and Bennett 1983, Willis 1985). In general, nociceptive primary afferents respond to noxious stimuli with conduction velocities in the range of A delta and C fibers. Analysis of the fiber population in a human cutaneous nerve indicate that there are approximately 3-5 times more C fibers than A delta fibers, though presumably not all of these fibers are nociceptors (Ochoa and Mair, 1969).

There are two major classes of cutaneous nociceptors which differ in their response characteristics (Dubner and Bennett 1983, Willis 1985). The first class are the A delta high threshold mechanoreceptors (HTM). These nociceptive fibers respond to noxious mechanical stimuli, but not generally to chemical or thermal stimuli.

A second major class of cutaneous nociceptors are the C fiber polymodal nociceptors. These fibers respond to thermal, mechanical and chemical noxious stimuli. Although other classes of cutaneous nociceptors have been described, these have not been as well characterized. Included in this group are mechanothermal and thermal receptors which may transmit nociceptive information as well (Dubner and Bennett 1983, Willis 1985).

In addition to specialization in terms of sensory responsiveness, recent evidence suggests that primary afferents may have a concordant degree of specialization in neurotransmitters secreted from central terminals located in the dorsal horn. Kuraishi, et al. (1985) reported that application of noxious pinch resulted in significant increases in immunoreactive substance P in dorsal horn superfusates, with no change in immunoreactive somatostatin. Conversely, application of noxious heat resulted in dramatic increases in somatostatin without changes in superfusate levels of substance P. Thus, primary nociceptive afferents appear to exhibit uniqueness both in terms of sensory responsiveness and in terms of released neurotransmitter.

The response of free nerve endings to a suprathreshold stimulus is believed to be similar to other forms of receptor transduction, with the development of a local non-propagated receptor potential exceeding the threshold for an action potential discharge. The action

potential, as a signal for the occurrence of tissue damage, is then transmitted along the primary nociceptive afferent to the dorsal horn of the spinal cord.

Primary afferents fan out as they enter their cutaneous receptive fields. As compared to C fibers, A delta fibers generally have a greater number of receptive fields spread out over a greater surface area (Burgess and Perl 1973). Although free nerve terminals lack morphological specialization as compared to other forms of cutaneous receptor types (e.g. Pacinian corpusles), they are believed to possess some degree of biochemical specialization in terms of membrane receptors and stored neuromediator substances. Interestingly, ultrastructural studies have demonstrated that free nerve endings contain agranular vesicles (Tervo and Palkama 1978, Hoyes and Barber 1976, Byers 1984, Arwill, et al. 1973), which are thought to contain agents which may be functionally involved in the modulation of inflammatory responses to tissue damage (Yaksh and Hammond 1982).

Peripheral opiate receptors located on primary afferents are logical targets for circulating opioid peptides. Two lines of evidence support the existence of opiate receptors on peripheral nerves and their involvement in pain modulation. First, a number of investigators have demonstrated opiate binding sites on peripheral nerves (Atweh, et al. 1978, Young, et al. 1980, Laduron 1984a,b, Hiller, et al. 1978, Fields, et al. 1980). Secondly,

pharmacological studies have demonstrated opiate-induced analgesia via peripheral mechanisms (Ferreira and Nakamura 1979a,b,c, Ferreira, et al. 1982, Rios and Jacob 1982, 1983, Bentley, et al. 1981, Randich and Maixner 1984, Hargreaves and Joris unpublished observations 1986). Thus, circulating B-End may modulate pain via activation of peripheral opiate receptors located on primary nociceptive afferents.

1-4.c. Ascending Transmission of Pain

The dorsal horn of the spinal cord contains four major components related to the processing of pain; central terminals of afferent fibers, interneurons, projection neurons and descending neurons. The first component, the primary nociceptive afferents, enter the dorsal horn of the spinal cord via the lateral aspect of the tract of Lissauer. Both A delta and C fibers terminate in lamina I, IIa, V, and X (Dubner and Bennett 1983, Willis 1985). Rexed's lamina I corresponds to the marginal layer, lamina II to the substantia gelatinosa and lamina V to the ventral nucleus proprius. Nociceptive afferent fibers have been proposed to utilize a variety of neurotransmitters including substance P, somatostatin, choleycystokinin, and calcitonin gene-related peptide (Yaksh and Hammond 1982, Rosenfield, et al. 1983).

The second component, the interneurons, consist of two major sub-types, the islet cell and the stalked cell. The islet cell is found throughout lamina II and is thought to be an inhibitory interneuron possibly employing GABA or enkephalin as neurotransmitters (Dubner and Bennett 1983, Willis 1985). The stalked cells are found primarily at the junction of lamina I/II and have been proposed to be excitatory interneurons conveying nociceptive output from primary afferents to second order neurons located in lamina I (Dubner and Bennett, 1983, Willis 1985). The activity of the interneurons themselves may be regulated by descending serotinergic and noradrenergic neurons (Dubner and Bennett 1983). Thus, the interneurons play a critical role in conveying and probably modulating nociceptive signals from the first order to the projection neurons.

The third component of the dorsal horn is the projection or second order neurons. The major projection system for pain is the spinothalamic tract (STT), which is formed predominately from neurons in lamina I and V. The second order neurons forming the STT can be activated by painful as well as by innocuous input.

Fibers from these second order neurons cross to the contralateral side of the spinal cord and project rostrally as the lateral STT. Axonal collaterals of the STT terminate in the rostral medullary reticular formation and the periaquaductal grey (see 1-4.d). The STT fibers themselves terminate in the thalamus. Fibers originating

from neurons located in lamina I and V terminate in the ventral posterior lateral (VPL) nucleus of the thalamus. Fibers from the STT also terminate in other thalamic nuclei (Willis 1985). However, terminations of the STT in the VPL retain somatotropic organization, while terminations in other thalamic nuclei do not. These observations indicate that the STT is a major projection system conveying nociceptive output from the dorsal horn to the thalamus.

Four other projection systems have been implicated in the rostral transmission of pain. The spinoreticular tract originates in dorsal horn lamina VII and VIII and terminates in medullary nuclei (Willis 1985). The spinoreticular tract may be involved in motivational-affective aspects of pain and may activate endogenous analgesic systems (see below). The spinomesencephalic tract originates in lamina I and V and terminates in mesencephalic nuclei such as the lateral periaquaductal grey. The spinomesencephalic tract may participate in both rostral transmission of pain and in activation of descending pain suppression systems. The spinocervical tract originates in lamina III, IV and V and terminates in the lateral cervical nucleus. The fourth spinal projection system is the post-synaptic dorsal column pathway. This pathway primarily originates in lamina III and IV, ascends in the ipsilateral dorsal funiculi and terminates in the dorsal column nuclei.

1-4.d. Descending and Segmental Pain Suppression Systems

This section reviews the neuroanatomical circuitry involved in endogenous pain suppression systems which act to modulate the perception of pain. Although the concept of an "endogenous analgesic system" may appear exotic, it is merely another example of the ability of the CNS to regulate incoming sensory information. For example, several well characterized systems are known to modulate acoustic, olfactory and visual sensory input, often at the level of the peripheral receptor or the first few synapses (Carpenter and Sutin 1984). Regulation of sensory input can originate in higher levels and is termed a descending or centrifugal control, since the primary site of sensory modulation probably occurs in the first few synapses in the spinal cord (Dubner and Bennett 1983). In addition, local or segmental modulation may arise in the spinal cord. Opiate receptors are located at all known levels of the endogenous pain suppression systems. This fact underlies the analgesic potency of opioids and exogenous opiates, since their administration results in activation of the endogenous pain suppression systems at all levels - producing a multiplicative analgesic effect (Yeung and Rudy 1980b).

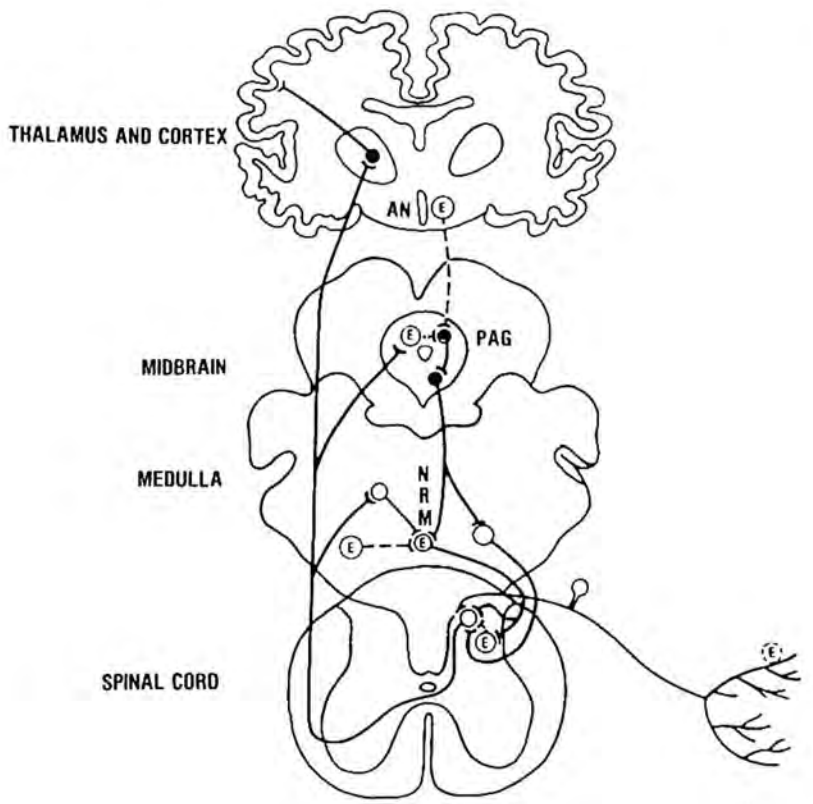
The current neuroanatomical model of endogenous pain suppression systems has been recently summarized by Bausbaum and Fields (1984) and can be considered as a three

level system (Fig. 1). The top, or most rostral level is at the periaquaductal grey (PAG). The PAG plays a critical role in integrating information from cortical and brainstem regions with incoming nociceptive signals. The PAG receives nociceptive input from axonal collaterals of the STT and terminal fibers from the spinomesencephalic tract. In additon, the PAG contains enkephalin and dynorphin interneurons and receives terminals containing B-END which project from the arcuate nucleus of the hypothalamus. These endogenous opioid peptides are thought to activate (probably by a disinhibition) descending fibers from the PAG which ultimately suppress pain transmission. This conclusion is strengthened by studies which indicate that microinjection of opiates into the PAG has been demonstrated to produce a profound analgesia (Yaksh and Rudy 1976, Yaksh, et al. 1976, Yeung and Rudy 1980a, Oyama, et al. 1980, Hosobuchi and Li 1978). Interestingly, the descending fibers from the PAG do not appear to utilize opioid peptides as transmitter substances, but rather employ serotonin and neurotensin as synaptic communicators. Descending fibers from the PAG terminate in rostral medullary nuclei.

The rostral medullary nuclei comprise the middle level of the pain suppression system and include the nucleus raphe magnus (NRM) and adjacent medullary reticular nuclei (Fig. 1). In addition to descending fibers from the PAG, the rostral medullary nuclei also receive ascending

Figure 1. This figure presents the known hierarchy of endogenous neural pain suppression systems. The periaquaductal grey (PAG) receives peripheral nociceptive information from axonal collaterals of the spinothalamic tract and terminal fibers of the spinomesencephalic tract and integrates this with signals from higher brain centers (not shown). The PAG contains endogenous opioid interneurons (symbolized with the letter ("E") and B-END terminals from projections in the arcuate nucleus (AN) of the hypothalamus. Descending fibers from the PAG terminate at several nuclei in the rostral medulla, only the nucleus raphe magnus (NRM) is shown. The rostral medulla also contains endogenous opioid interneurons. From the medulla, descending fibers travel in the dorsolateral funiculus of the spinal cord, where they terminate in the dorsal horn, possibly on endogenous opioid interneurons ("E") or directly on the projection neurons. Thus, the PAG integrates information from the periphery and higher brain centers and, via a medullary link, acts to suppress the transmission of pain signals in the dorsal horn of the spinal cord. Note that endogenous opioids work at all known levels to activate this system possibly including opiate receptors in the free nerve terminal out in the primary nociceptive afferent nerve fiber. Circulating opioids may activate this system by occupying opiate receptors located either in the CNS or in the periphery.

POTENTIAL INTERACTIONS BETWEEN NEURONAL AND HUMORAL PAIN CONTROL SYSTEMS



nociceptive input via axonal collaterals from the STT and terminal fibers from the spinoreticular tract. The medullary reticular nuclei contain enkephalin and dynorphin interneurons and microinjection of opiates into this region produces potent behavioral analgesia. Fibers project from the medullary reticular nuclei via the dorsolateral funiculus to the dorsal horn of the spinal cord. These fibers are believed to be predominately serotonergic or noradrenergic.

The dorsal horn of the spinal cord is the lower, or most caudal level of the pain suppression system (Fig. 1). Descending fibers from the rostral medullary nuclei may modulate the transmission of nociceptive information either directly, by inhibitory synaptic contacts with the projection neurons (e.g. STT neurons), or indirectly, via activation of inhibitory spinal interneurons or possibly, by a combination of the two. Both enkephalin (in lamina I, II and V) and dynorphin (in lamina I) interneurons have been reported in the dorsal horn. The observations that intrathecal injections of opiates produce profound analgesia in both animal and clinical models of pain points to the physiologic importance of both opioid peptides and opiate receptors in modulating pain transmission at the spinal cord level.

In a series of studies evaluating the antinociceptive activity of intrathecal opiates, with differing receptor selectivities, Schmaus and Yaksh (1984)

demonstrated that opiate receptor sub-types differ in their ability to block behavioral responses to pain of cutaneous thermal as compared to chemical visceral types. Antinociception for cutaneous thermal pain (hot plate model) was observed following intrathecal administration of relatively selective mu opiate agonists, relatively selective delta agonists and mixed mu/delta agonists. In contrast, little to no thermal antinociceptive activity was observed following intrathecal administration of kappa agonists or sigma agonists. A different pattern for antinociceptive activity was observed using visceral chemical pain (acetic acid stretch model). In this model, relatively selective mu agonists were again effective, as were mu/delta mixed agents, but, in addition, kappa agonists were also antinociceptive. In contrast, delta selective agents had little activity even at doses producing non-selective effects. The results indicate that different modalities of pain can be differentially modulated by opiate receptor sub-type activity. These pharmacological studies suggest the functional presence of distinct spinal opiate systems capable of selectively modulating different forms of noxious stimuli.

Taken together, the obvious neuroanatomical complexity underlying the detection and processing of painful stimuli indicates that the perception of pain is not a simple function of tissue damage and that it can be modified at any of several possible target sites by opioid

peptides, derived from either neuronal or endocrine origins.

1-5. Proposed Mechanism of Action For Analgesic Properties of Pituitary B-END

1-5.a. Retrograde Flow

Three hypotheses have been advanced to describe the mechanism for analgesia which may result from pituitary release of B-END. First, B-END may gain access to receptors in the CNS by retrograde flow from the pituitary to the hypothalamus via the hypophyseal portal circulation. The retrograde flow hypothesis has been recently reviewed by Page (1982), Mezey and Palkovits (1982) and Flerko (1980). While the predominant pattern of portal blood flow is brain-pituitary, several lines of evidence suggest the existence of a parallel pituitary-brain vascular pathway. These studies utilized either anatomical or direct assessment techniques.

Anatomical studies focused either on venous drainage paths from the adenohypophysis using vascular casts, or directly evaluated vascular dynamics (Bergland and Page 1978, Ambach, et al. 1978, Torok 1962). The studies concluded that: 1) intracarotid dye injections demonstrated rostral direction of flow; 2) vessels of the infundibular stalk plexus, subependymal plexus and

neurohypophysis conveyed this circulation; and 3) bidirectional flow appeared probable due to lack of sufficient venous drainage from the cavernous sinus.

It should be noted, however, that the hypothesis of retrograde flow is controversial owing to the extreme difficulty involved in studying the hypophyseal portal circulations. For example, photography of the dorsal surface of the pituitary in young craniectomized pigs under general anesthesia revealed retrograde flow within the pars nervosa but an absence of retrograde flow from the pars distalis (Page, 1983). In addition, Doppler studies of long portal vessels on the anterior surface of the pituitary stalk in anesthetized Rhesus monkeys detected predominant hypothalamic-pituitary flow, with cessation of net flow occurring during a valsalva manuever (Antunes, et al. 1983).

Direct measurement of hormones in portal blood have also produced variable results with respect to evidence supporting the concept of retrograde flow. The work of Oliver, et al. (1977) demonstrating the dramatic fall in portal blood levels of ACTH after hypophysectomy has already been described. There appears, however, to be a species or time related variability in this finding since Wardlaw noted little change in portal blood levels of ACTH in monkeys immediately after hypophysectomy (Wardlaw, et al. 1980), and yet Schlachter, et al. (1983) reported significant reduction in CSF levels of B-END in humans

after hypophysectomy. Functional evidence of retrograde flow has been advanced by Dorsa, et al. (1979) who demonstrated that intrapituitary injection of neurotensin resulted in hypothermia, which could be blocked by prior pituitary stalk section. In another series of studies, Mezey, et al. (1978, 1981) and Mezey and Palkovits (1982) determined brain uptake of $^{3}H\text{-}ACTH_{4-9}$ following intrapituitary, intravenous, and intrasellar injections. Intrapituitary injection of the labelled peptide (0.1 ul injected over 2 minutes) resulted in an accumulation in the hypothalamus which was greater than that observed following intravenous or intrasellar (i.e., placed under the dura surrounding the pituitary) injections. Further, retrograde transport of labeled ACTH following pituitary injection was shown to be dependent upon an intact portal system. Intrapituitary injection of ^{3}H-B-LPH also resulted in CNS accumulations, especially in the hypothalamus, pre-optic and septal areas (Mezey, et al. 1981) Together, these lines of evidence, advanced by independent research strategies, suggest the functional existence of retrograde flow, although the issue cannot be considered as resolved.

The ability of B-END to produce analgesia when placed into the hypothalamus is well established. B-END microinjected into either the anterior hypothalamus or medial pre-optic areas produced potent antinociception (Tseng, et al. 1980). In addition, microinjection of cyclazocine or morphine (2.6 ug in 0.5-1.0 ul over 80-120

sec.) into the dorsomedial hypothalamus activated naloxone-sensitive, descending analgesia systems which suppressed neural and behavioral responses to peripheral administration of bradykinin (Satoh, et al. 1985, Kawajiri and Satoh 1985). Moreover, electrical stimulation of the dorsomedial hypothalamus activated descending pain suppression systems as evidenced by behavioral antinociception (Rhodes and Liebeskind 1978) and inhibition of spinal dorsal horn neuron responses to noxious stimulation (Carstens 1982, Carstens, et al. 1983). Thus, hypothalamic sites possessing opiate receptors and the capability of eliciting analgesia are positioned directly above the anterior pituitary and in the pattern of retrograde flow.

1-5.b. Permeability Across The Blood-Brain-Barrier

The second hypothesis proposes that B-END released from the pituitary enters the systemic circulation and subsequently stimulates CNS opiate receptors by crossing the BBB. Initial studies suggesting that endorphins did not cross the BBB used the Oldendorf method, which detects only highly permeable substances. Interestingly, this method predicts that morphine does not enter the CNS (Cornford, et al. 1978). Subsequent studies have utilized methods allowing detection of lower permeability coefficients. Rapoport, et al. (1980), reported that a B-

END analog exhibited moderate, though significant, CNS entry when injected intravenously in rats. In addition, Houghton, et al. (1980), demonstrated that i.v. administration of a B-END analog produced a slow accumulation of radioactivity in rabbit CSF and that 75% of this radioactivity coeluted with authentic B-END on a sephadex G-50 column. These data indicate that B-END can enter the CNS from the vascular compartment.

The work of Pechura, et al. (1982, personal communication, 1984, Hayes, et al. 1985), demonstrated that the permeability of the BBB changes with stress. As described earlier, they observed that increased permeability across the BBB occurs in response to stress, allowing increased access of blood-borne substances to the CNS. Interestingly, this change may be hormonally regulated, since hormones of the pituitary-adrenal axis (ACTH, MSH and glucocorticoids) altered BBB permeability (Sankar, et al. 1981, Rundman and Kutner 1978, Long and Holaday 1985). Accordingly, circulating B-END may gain access to CNS target sites by crossing the BBB and this occurs at increased rates during stress.

1-5.c. Peripheral Opiate Receptors

The third hypothesis proposes that the target site for pituitary B-END are peripheral opiate receptors capable of modulating nociception. Although it is widely accepted

that opiates produce analgesia through action on the CNS (Jaffe and Martin 1985), several lines of evidence support a peripheral site of action as well.

The first line of evidence supporting this view was provided by the early work of Lim, et al. (1964). The experimental design employed a "vasoisolated but innervated spleen", where a recipient dog's splenic blood flow was cross-perfused to a donor dog (Lim, et al. 1964). This model allowed for a separation of central, versus peripheral, site of action of drugs. While the authors conclude that opiates act only centrally, examination of their data reveals that morphine (1 mg/kg i.v.) clearly suppressed bradykinin-evoked splanchnic nerve activity through a peripheral action (Fig. 9-II, Lim, et al. 1964) In addition they reported that propoxyphene has a peripheral analgesic effect when administered locally into the splenic circulation in doses one-half as large as the systemic ED_{50} (Lim, et al. 1964). Despite their actual findings, the early interpretations of Lim, et al. (1964) focused subsequent research on delineating the mechanisms for central actions of opiates, while potential peripheral mechanisms for opiate analgesia remained largely ignored.

An additional line of evidence more clearly supports a peripheral site of action for opiate analgesia. Microinjection of naloxone into either intrathecal or intraventricular spaces attenuated, but did not abolish, the antinociceptive effects of systemically administered

morphine in animals (Yeung and Rudy 1980b, Yaksh and Rudy 1977, Vigouret, et al. 1973). Conversely, peripheral administration of naloxone completely blocks morphine antinociception. These findings suggest that opiates exert analgesic actions through activation of receptors located outside of the CNS.

The existence of opiate receptors outside of the CNS has been known for some time. B-END and opiate binding sites have been located in the gastrointestinal tract, the vas deferens, Merkel cells, t-lymphocytes, adrenal medulla, liver, retina and in the cardiovascular system (Medzihradsky 1976, Willette and Sapru 1982, Schweigerer 1983, Leslie, et al. 1980, Dave, et al. 1985a). However, only recently has evidence been advanced to support the localization of opiate receptors on peripheral nerves. Several investigators have demonstrated opiate receptor binding sites in peripheral nerves (Atweh 1978, Young, et al. 1980, Laduron 1984a,b, Hiller, et al. 1978, Fields, et al. 1980). These peripheral opiate binding sites displayed properties of opiate receptors such as saturability and selectivity. Moreover, reduction in binding occurred if the animal was pretreated with naloxone in vivo, prior to the in vitro binding assay (Laduron 1984b). Opiate receptors on peripheral nerves appeared to undergo axonal transport towards the periphery since placement of a ligature was found to cause a dramatic increase in the density of opiate binding sites proximal to the ligature

(Young, et al. 1980, Laduron 1984a,b). Opiate receptors have also been detected in peripheral nerves near the dorsal root ganglion of the spinal cord (Hiller, et al. 1978, Fields, et al. 1980). The existence of opiate receptors on peripheral nerves provides a neurophysiological basis for the investigation of their potential role in the development of analgesia.

Ferreira and co-workers have reported that activation of peripheral opiate receptors blocks the hyperalgesia following administration of prostaglandin E_2 (PGE_2) in the rat paw (Ferreira and Nakamura 1979 a,b,c, Ferreira 1982). Hyperalgesia is a basic component of inflammation and is believed to be mediated by the release of prostaglandins (Ferreira 1972, Greaves, et al. 1971, Lembeck, et al. 1976). Prostaglandins produced in response to tissue injury have been shown to modulate many of the signs of inflammation including sensitization of peripheral nociceptors (Ferreira 1972, Greaves, et al. 1971, Lembeck, et al. 1976, Collier and Roy 1974a,b,c, Kalix 1979).

The mechanism proposed by Ferreira for peripheral analgesic actions of opiates is through the inhibition of prostaglandin-stimulated adenylate cyclase (Ferreira and Nakamura 1979 a,b,c). This conclusion is based on reports that opiates inhibit prostaglandin-stimulated adenylate cyclase under a variety of experimental conditions (Collier and Roy 1974 a,b,c, Haveman and Kuschinsky 1978 a,b, Lembeck and Beubler 1979).

In Ferreira's model, intraplantar administration of 100 ng of PGE_2 into a rat paw produces a long lasting hyperalgesia as demonstrated by pain responsiveness to pressure. In this model, analgesia was defined as an increased latency to withdraw the hindpaw. In a typical experiment, a bilateral hyperalgesia was produced by intraplantar administration of PGE_2 to both hindpaws. Subsequent intraplantar administration of morphine sulfate (10 ug) to one hindpaw produced a unilateral analgesic effect in that paw (Ferreira and Nakamura 1979 b,c); analgesia was not observed in the contralateral paw. Based on these findings, a CNS site of action for the opiate can be excluded since drug diffusion into the CNS would have produced a generalized analgesia. The absence of a bilateral analgesia, following unilateral administration of morphine, provides evidence supporting a local analgesic site of action.

Bentley, et al. (1981) have confirmed Ferreira's observations using a model of chemically-induced inflammatory pain, the acetic acid writhing test. They observed that: 1) locally administered morphine is 100 times more potent in producing antinociception as compared to morphine administered i.v. or i.c.v.; 2) the onset of morphine action was too short (1-2 minutes) to permit absorption and distribution into the brain; and 3) stereospecificity was observed since levorphanol was 8

times more potent than its inactive isomer, dextromethorphan.

Results derived from other models of pain have also indicated peripheral involvement of opiates in modulating nociception. For example, Inoki, et al. reported that locally administered morphine inhibited the release of bradykinin into perfusates from either tooth pulp or rat paw following electrical nerve stimulation (Inoki, et al. 1973, 1977). In addition, quaternary analogs of opiates poorly, if at all, penetrate the blood brain barrier and yet possess analgesic properties (Smith, et al. 1982, Ferreira and Rae 1984). Finally, intra-jugular administration of a met-enkephalin analog produced a significant antinociception which was blocked by bilateral vagotomy (Randich and Maixner 1984). The time course of this effect (maximal 15 seconds after injection) was interpreted to exclude a CNS site of action.

In addition to modifying pain, opiates may modulate another component of inflammation, increased capillary permeability and vasodilation. Plasma extravasation produced by nerve stimulation is widely employed as a model for assessing changes in capillary permeability. In this model, Evans blue dye, which tightly binds to plasma albumin, is injected intravenously and serves as a marker for measuring the accumulation of extra-vascular albumin in regions with increased capillary permeability. Subsequent peripheral nerve stimulation produces an increased

capillary permeability as measured by accumulation of Evans blue dye in the innervated cutaneous fields.

Plasma extravasation is probably produced by release of substance P from peripheral nerve fibers. The release of substance P from peripheral C-fibers has been implicated in mediating increased capillary permeability, since: 1) substance P was shown to be released following electrical stimulation of peripheral nerve (White and Helme 1985); 2) extravasation was produced by local administration of substance P (Lembeck and Holzer 1979); 3) extravasation was blocked by pretreatment with substance P antagonists (Lembeck, et al. 1982); 4) extravasation was blocked by neonatal administration of capsaicin, a substance P depleter (Lembeck and Holzer 1979, Morton and Chahl 1980); 5) extravasation was unaffected by guanethidine-induced sympathectomy (which inactiviates sympathetic C fibers) (Lembeck and Holzer 1979); and 6) a requirement of electrical stimulation was that it must be of sufficient intensity to activate C fibers (Hinsey and Gasser 1930). These findings indicate that increased capillary permeability resulting from peripheral nerve stimulation is probably due, at least in part, to release of substance P from nerve endings of primary C fiber afferents.

The administration of opiates results in a dramatic blockade of neural-induced plasma extravasation. The opiate blockade is reversed by naloxone (Lembeck and Holzer

1979, Morton and Chahl 1980, Bartho and Szolcsanyi 1981, Lembeck, et al. 1982, Smith and Buchan 1984). Both morphine (Smith and Buchan 1984) and ethlyketocyclazocine (Bartho and Szolcsanyi 1981, Lembeck, et al. 1982) were shown to be potent in this model. It is of interest to note that similar effects of opiates on inhibiting substance P release from central terminals of primary afferents have also been reported (Jessell and Iverson 1977, Yaksh, et al. 1980). A physiologic interaction between peripheral substance P and receptors for opiates is further supported by the findings that: 1) morphine inhibited immunoreactive-substance P release from peripheral sites in vivo (Brodin, et al. 1983); and 2) opiates had no effect on plasma extravasation due to locally administered substance P (Lembeck, et al. 1982, Smith and Buchan 1984). Thus, opiates appear to act on terminals of nociceptive neurons to modulate peripheral responses to tissue damage, including pain and increased vascular permeability.

A non-specific local anesthetic mechanism in the peripheral action of opiates does not appear to be the case since: 1) peri-neural administration of morphine proximal to the receptive field did not alter compound action potentials, neurogenic plasma extravasation or post-operative pain (Yuge, et al. 1985, Bullingham, et al. 1983, Smith and Buchan 1984); and 2) morphine did not alter

cyclic AMP-induced hyperalgesia (Ferreira and Nakamura 1979 a,b).

Taken together, the above findings firmly support the hypothesis that blood-borne B-END modifies nociception through actions on peripheral opiate receptors.

1-6. Evidence Implicating Pituitary B-END and Analgesia

1-6.a. Stress-Induced Analgesia and Stimulation of Pituitary Release

Numerous findings indicate that certain environmental stimuli are capable of producing an antinociceptive state. This diminution in behavioral nociception has been termed stress-induced analgesia (SIA). Many, but not all, stressors elicit an SIA, including both noxious (e.g., electrical shock) and non-noxious (e.g., immobilization) stimuli. This section reviews, from a behavioral perspective, evidence demonstrating activation of endogenous pain suppression systems following aversive environmental stimuli.

Although known pain suppression systems have been reviewed from an anatomical perspective (1-4.d.), it is still unclear to what extent they participate in the various forms of SIA. Although the precise mechanisms mediating SIA have not been identified, experimental evidence indicates that several possible mechanisms exist

and may operate concurrently. For this reason, a descriptive system for classifying SIA has been developed. According to this system, SIA is divided into four categories, designated neural opioid, neural non-opioid, hormonal opioid and hormonal non-opioid (Watkins and Mayer 1982, Terman, et al. 1984, Watkins, et al. 1982, Basbaum and Fields 1984). Opioid mediation is generally determined by a naloxone or naltrexone challenge. As implied by the terms describing the categories, not all forms of SIA are reversed by opiate antagonists. A hormonal component is generally indicated by the results of experiments which involve gland ablation, such as hypophysectomy. For example, hypophysectomy is reported to block immobilization-induced antinociception (Amir and Amit 1978b), but has no effect when the stimulus is prolonged continuous electrical shock (Terman, et al. 1984). It is important to note that these classification criteria are not absolute and appear to reflect only gross differences among the physiological mechanisms which result in SIA.

Different forms of stress produce different forms of SIA. Factors in determining the mechanism(s) which predominate in the development of an endogenous antinociception include the type and intensity of the stress, its duration and the body region to which the stress is applied (Bodnar, et al. 1979a, Watkins and Mayer 1982, Terman, et al. 1984, Lewis, et al. 1980). The finding that environmental stressors differed in their

ability to stimulate the pituitary-adrenal axis (Mueller 1981, Mason, et al. 1976, Vernikos-Danellis and Heybach 1980), provides biochemical evidence that physiologic responses to stressors do indeed vary. Accordingly, the relative participation of pituitary B-END in SIA, as compared to other putative pain-suppression mechanisms, probably varies under different experimental conditions.

1-6.b. Effect of Pituitary Interventions on SIA

Several investigators have reported findings which together demonstrate that stressors can activate both pituitary B-END release and hormonal-dependent forms of SIA (Mueller 1981, Akil, et al. 1982, Krieger, et al. 1977b, Bodnar, et al. 1979ab, Watkins and Mayer 1982, Amir and Amit 1978ab, Akil, et al. 1976). It should be emphasized, however, that these findings only demonstrate an association between circulating B-END and SIA. Studies designed to determine a cause and effect relationship between pitutiary secretion of B-END and SIA have involved interventions which prevent either B-END release or B-END action.

Two forms of pituitary intervention have been commonly used to prevent B-END release; hypophysectomy and glucocorticoid administration. Numerous reports have indicated that hypophysectomy abolishes the SIA produced by a variety of stressors (immobilization, foot shock (some

forms) and insulin-induced hypoglycemia) (Bodnar, et al. 1979abc, Amir and Amit 1978b, Akil, et al. 1976, Millan, et al. 1980, Pomeranz and Cheng 1977, Ramabadran 1982). The effect of hypophysectomy on SIA was not due to a generalized motor deficit since it did not alter hot plate escape behavior in non-stressed rats (Amir and Amit 1978b, Ramabadran 1982). Rather, removal of the pituitary appears to block the ability of certain stressors to produce antinociception.

Glucocorticoids provide a less traumatic and specifically more selective inhibiton of pituitary B-END secretion. Like ACTH, the secretion of B-END is under negative feedback inhibition by adrenal corticosteroids. Administration of dexamethasone, a synthetic corticosteroid which blocks the secretion of B-END, is widely reported to block forms of SIA which are pituitary dependent (Lewis, et al. 1980, Bodnar, et al. 1979c, Gaiardi, et al. 1983, Cheng, et al. 1979, Marek, et al. 1982, 1983). Conversely, adrenalectomy, which results in a compensatory hypersecretion of pituitary B-END, potentiated these forms of SIA (Marek, et al. 1982, 1983). Thus, treatments which enhance pituitary B-END secretion can facilitate SIA, whereas treatments which inhibit pituitary B-END release inhibit SIA.

1-6.c. Effect of Opiate Interventions on Pituitary-Dependent SIA

The functional relationship between SIA and pituitary B-END can be interpreted from the results of several studies which have employed opiate drugs and their antagonists. However, conclusions must be viewed with caution since these interventions do not discriminate between hormonal or neural opioid mechanisms for SIA. Therefore only those studies utilizing stressors whose SIA is abolished by hypophysectomy appear relevant for the present discussion. It is a uniform observation that these forms of SIA are blocked by naloxone pretreatment (Lewis, et al. 1980, Bodnar, et al. 1979c, Gaiardi, et al. 1983, Cheng, et al. 1979, Marek, et al. 1982, 1983). Moreover, chronic SIA was shown to produce a behavioral tolerance resembling that caused by chronic opiate administration (Madden, et al. 1977, McGivern, et al. 1983, Nabeshima, et al. 1983). Further, a cross tolerance with morphine has been reported with several forms of pituitary dependent SIA (Chesler and Chan 1977, Isumi, et al. 1983, Nabeshima, et al. 1983). These results indicate that pituitary-dependent SIA exhibits characteristics which are consistent with a functional involvement of endogenous opioid peptides.

1-6.d. Problems in Interpreting Stress Studies

Together, the observations discussed above suggest that certain stressors produce an SIA which is dependent

upon a hormonal opioid component and supports the hypothesis that pituitary B-END modulates antinociception. Until recently, this hypothesis could only be tested indirectly through application of stressful stimuli. It is important to recognize that stress is non-specific, in that a variety of physiologic systems are activated and yet only a few are probably relevant to the observed anti-nociception. Stress is a poor intervention to employ in studies testing specifically for pituitary B-END analgesia since: 1) it is non-selective; 2) different stressors vary in their ability to stimulate pituitary B-END secretion; 3) stress may activate other forms of SIA; and 4) the stress itself may increase population variance in subsequent behavioral testing. To directly test this hypothesis, a potent compound which selectively stimulates pituitary secretion of B-END is required. The best compound available for this purpose is corticotropin releasing hormone (CRH).

1-7. Corticotropin Releasing Hormone

1-7.a. Historical Review

The search for a CRH spans nearly 30 years. Slusher and Roberts first reported an ACTH releasing extract from hypothalmi in 1954. Additional evidence supporting this concept was advanced in 1955 by both

Guillemin and Rosenberg and Saffran and Schally. CRH was the first releasing factor to be recognized, yet its structual identity has been only recently elucidated. Despite massive efforts to purify it, isolation of CRH proved difficult due to lack of a sensitive bioassay, peptide lability and the very small amounts present in brain. Finally in 1981, Vale, et al. reported the isolation and identification of a 41 amino acid peptide possessing properties of a CRH. This peptide was tentatively identified as a releasing factor (i.e., CRF), subsequent to findings demonstrating its physiologic role as a releasing hormone (i.e., CRH).

1-7.b. Anatomical Distribution of CRH

Several lines of anatomical, biochemical, physiological and pharmacological evidence support the proposition that Vale's peptide is the endogenous corticotropin releasing hormone. The anatomical distribution of immunoreactive CRH is consistent with its role as a hypothalamic hormone. As predicted by earlier studies evaluating the effects of hypothalamic lesions on pituitary-adrenal axis activity (Makara, et al. 1981), high concentrations of immunoreactive CRH have been visualized in cell bodies in the paraventricular nucleus with fibers projecting laterally and ventrally to the median eminence (Swanson, et al. 1983, Merchenthaler, et al. 1983).

Moreover, increased densities of immunoreactive CRH staining were evident in hypophysectomized or adrenalectomized animals (i.e., under conditions when glucocorticoid negative feedback is absent (Merchenthaler, et al. 1983, Paull and Gibbs. 1983). Immunoreactive CRH is reported to be present in other areas of the CNS, including basal telencephalon, cerebral cortex, medulla oblongata, dorsal root ganglion and spinal cord (Swanson, et al. 1983, Schipper, et al. 1983, Skofitsch, et al. 1985). However, staining densities of these other areas do not change following adrenalectomy (Swanson, et al. 1983).

Importantly, immunoreactive CRH has been demonstrated to be present in hypophyseal portal blood (Gibbs and Vale 1982) at concentrations near the dissociation constant for CRH receptors on pituicytes (Wynn, et al. 1983). Moreover, portal blood and median eminence levels of CRH both increased following hemorrhagic stress and adrenalectomy (Plotsky and Vale 1984, Suda, et al. 1983); conditions which accelerate pituitary corticotroph function. By contrast, median eminence levels of immunoreactive CRH fell by 90% after lesion of the paraventricular nucleus. The results indicate that immunoreactive CRH is present and responsive in an anatomical context consonent with its role as a regulator of pituitary corticotroph function.

1-7.c. Biochemistry of CRH

Biochemical evidence suggesting that Vale's CRH is indeed a physiologic corticotroph regulator is based on two independent lines of investigation. First, the initial structural identification of the 41 amino acid peptide followed the classical approach of purification, sequence analysis and synthesis from ovine hypothalamic extracts (Vale, et al. 1981, Spiess, et al. 1981). This was followed by a second classical purification procedure using rat hypothalamii, which resulted in the identification of a rat CRH differing 7 amino acids from the ovine CRH (Rivier, et al. 1983). Thus, a peptide possessing predicted bioactivity was isolated from the appropriate tissue of origin.

The second biochemical approach identifying CRH used molecular biology techniques. In the first report from Numa's lab, Furutani, et al. (1983) developed a cDNA library from ovine hypothalamic mRNA and screened it for pre-pro-CRH mRNA by hybridization to a synthetic oligonucleotide probe for CRH, based on the structure of Vale's CRH. The identified ovine mRNA contained a nucleotide sequence which, following translation, produced the same 41 amino acid peptide previously identified by Vale, et al. Additionally, the pre-pro-CRH mRNA was shown to code for dibasic amino acid pairs on each side of the CRH and thus provide cleavage sites for the enzymatic liberation of CRH from its precursor. A second report from

Numa's lab identified the sequence for the human pre-pro-CRH gene (Shibahara, et al. 1983). Based on the gene sequence, the amino acid structure for human CRH is the same as the rat CRH previously identified by Vale's group (Rivier, et al. 1983). Thus, both chemical and molecular biology methods have provided independent verification of the structure of ovine, rat and human CRH.

1-7.d. Pharmacological/Physiological Properties of CRH

Several lines of physiologic evidence support the contention that these identified peptides are CRHs. It has been established that CRH dose-dependently stimulates pituitary corticotroph secretion of POMC derived peptides administered either in vivo or in vitro to rats, humans, sheep or dogs (Vale, et al. 1981, Bruhn, et al. 1984, Spiess, et al. 1981, Chan, et al. 1982, Rivier, et al. 1982a). Doses of CRH that are effective in vitro closely approximate the content of CRH in portal blood (as low as 10 pM) (Vale, et al. 1981, Spiess, et al. 1981, Gibbs and Vale 1982, Plotsky and Vale 1984). It is now well established that CRH-induced secretion of POMC derived peptides is Ca^{++} dependent (Vale, et al. 1981). In addition to CRH's immediate effects on stimulating secretion, CRH also causes an increase in POMC mRNA in corticotrophs (Bruhn, et al. 1984). Both in vivo and in vitro stimulatory effects of CRH are blocked by prior

administration of dexamethasone (Vale, et al. 1981, Rivier, et al. 1982a, Bruhn, et al. 1984, Bilezikjian and Vale 1983). This last point emphasizes the physiologic regulation of corticotroph function which must exist between releasing hormone stimulation and glucocorticoid feedback inhibition. Finally, immunoneutralization by administration of anti-CRH antiserum blocks 75% of the ACTH released in response to trauma, immobilization, formalin injection and ether stress. (Rivier, et al. 1982b, Linton, et al. 1985, Nakane, et al. 1985). Additionally, immunoneutralization blocks the compensatory hypersecretion of ACTH observed after adrenalectomy (Rivier, et al. 1982b). Thus, Vale's CRH possess physiologic properties which are entirely consistent with its role as the corticotropin releasing hormone.

A pharmacological approach supporting the contention that Vale's peptide is CRH is based on development of CRH antagonists. Rivier, et al. (1982b) developed analogs of CRH maximizing the alpha helical pattern. Incorporation of 10 amino acids, coupled with deletion of the N-terminal 8 amino acids, produced a peptide, termed alpha helical CRF_{9-41}, with low intrinsic agonist activity and with significant antagonist activity (Rivier, et al. 1984). Administration of alpha helical CRF_{9-41} blocked exogenous CRH-induced stimulation of ACTH, but had no effect on the actions of GHRH, GNRH or TRH (Rivier, et al. 1984). Importantly, alpha helical CRF_{9-41}

also blocked pituitary release of ACTH in response to ether stress and, moreover, suppressed the compensatory hypersectretion of ACTH in adrenalectomized rats (Rivier, et al. 1984). Thus, antagonists to Vale's peptide blocks the effects of endogenous CRH released under physiologic conditions.

Taken together, data from anatomical, biochemical, physiological and pharmacological studies provide compelling evidence that Vale's peptide is a CRH. Thus, Vale's peptide in either the ovine, or rat/human forms, will be referred to as CRH.

Interestingly, CRH possesses biological activity in addition to stimulation of the pituitary-adrenal axis. A spectrum of autonomic, endocrine and behavioral changes has been seen following administration of CRH. This gives rise to the speculation that CRH acts centrally presumably as a neurotransmitter, to initiate and integrate many of the physiologic responses to stress (Vale, et al. 1981). Beyond the anatomical evidence of the presence of CRH in nerve terminals of brain (Swanson, et al. 1983, Skofitsch, et al. 1985), several other lines of data support this premise.

Several endocrine effects are observed following central administration of CRH. Administration of CRH i.c.v. into sheep, monkeys and rats resulted in a delayed stimulation of the pituitary adrenal axis (Donald, et al. 1983, Kalin, et al. 1983c, Insel, et al. 1984). In

addition, CRH given i.c.v. to rats caused a dose related inhibition of both growth hormone and luteinizing hormone secretion (Rivier and Vale 1984ab); these effects were not observed in vitro indicating a central action of CRH (Vale, et al. 1981). Taken together, central administration of CRH, in either sheep, monkeys or rats, caused a delayed stimulation of the pituitary-adrenal axis; other endocrine changes appear to occur in rats but not Rhesus monkeys.

Autonomic changes are also observed following central administration of CRH. In a series of studies using rats, Brown and Fisher have demonstrated that i.c.v. administration of CRH produced dose related increases in mean arterial pressure, oxygen consumption and tachycardia, as well as increases in plasma levels of glucagon, epinephrine, norepinephrine and glucose (Fisher, et al. 1982, Brown, et al. 1982ab, Brown and Fisher 1983). These effects appear to be mediated via activation of the sympathetic nervous system since they were inhibited by pretreatment with the ganglionic blocker chlorisondamine and were unaffected by either hypophysectomy or adrenalectomy (Fisher, et al. 1982, Brown, et al. 1982b, Brown and Fisher 1983). Interestingly, these effects were not observed following peripheral administration of CRH (Brown, et al. 1982a, Brown and Fisher 1983). Although changes in mean arterial blood pressure, heart rate or plasma epinephrine were not observed in Rhesus monkeys, doses of CRH employed may have been too low to elicit these

responses (Insel, et al. 1984). In most instances then, central administration of CRH causes a delayed stimulation of the pituitary-adrenal axis and other endocrine and autonomic changes which appear to related to a neurotransmitter function of CRH in brain.

CRH is also active in producing autonomic and endocrine changes when administered peripherally. Intravenous administration of CRH resulted in a dose related hypotension, reportedly due to dilatation of the mesenteric circulation, in rats, dogs, monkeys and humans (Brown and Fisher 1983, Kalin, et al. 1983a, Grossman, et al. 1982). When administered to urethane-anesthetized rats, CRH produced both a hypotension and a bradycardia. The CRH-induced bradycardia appeared to be due to secretion of pituitary B-END, since it is blocked by naloxone, hypophysectomy and dexamethasone pretreatment (Kiang and Wei 1985).

Peripheral administration of CRH produces an immediate dose related stimulation of the pituitary-adrenal axis which is blocked by dexamethasone pretreatment (Vale, et al. 1981, Rivier, et al. 1982a, Kalin, et al. 1983ab, Donald, et al. 1983, Giguere, et al. 1982, Schulte, et al. 1982). The effect of peripherally administered CRH on other endocrine systems has also been evaluated. In sheep, CRH had no effect on plasma GH, LH, PRL, insulin, glucagon, met-enkephalin, angiotensin II, aldosterone or vasopressin (Donald, et al. 1983). By contrast, when given to

Cynomolgus macaques with transected pituitary stalks, CRH was reported to stimulate ACTH, GH and PRL release with no effect on plasma levels of either LH or TSH (Schulte, et al. 1982). Interestingly, naloxone inhibited the release of both GH and PRL in this model (Schulte, et al. 1983). It is unclear whether the lack of corticotroph selectivity exhibited by CRH is due to species differences, or to paracrine actions of corticotroph hormones (e.g. B-End), or simply reflects additional releasing properties of the peptide. The later is probably not the case since CRH is exquisitely selective when acting on rat, sheep or human pituitary; only corticotroph secretion is stimulated. Overall, the predominant effect following peripheral administration of CRH is stimulation of the pituitary-adrenal axis.

1-7.e. Behavioral Effects of CRH

In addition to its effects on autonomic and endocrine systems, CRH also has behavioral effects. Early, anecdotal reports, indicated that increases in motor activity followed i.c.v. but not i.v. administration of CRH (Brown, et al. 1982a). Subsequently, Sutton and co-workers evaluated the behavioral effects following i.c.v. administration of CRH to rats in a novel or familiar environment (Sutton, et al. 1982). CRH administered i.c.v. produced a significant dose-related increase in locomotor

activity in a familiar environment and a decrease in a novel environment (Sutton, et al. 1982). This effect of CRH was blocked by pretreatment with the CRH antagonist, alpha helical CRF_{9-41} (Britton, et al. 1986).

The effects of i.c.v. CRH on altering behavior in rats have been reportedly corroborated and extended to include a dose-related suppression of food intake (Britton, et al. 1982, Morley, et al. 1982a,b, Gosnell, et al. 1983ab). CRH suppressed food intake following starvation and its actions were unaffected by hypophysectomy (Morley, et al. 1982a,b). The strength of these findings, however, is weakened by the use of unblinded observers. This raises the concern that observer bias may have influenced the measured treatment effect.

Consistent with the findings in rodents, CRH causes situation-dependent behavior in monkeys. CRH given i.v. to 6 chair restrained monkeys produced increased struggling and visual exploration (Kalin 1983b). Conversely, when given i.v. to free ranging monkeys, CRH was associated with significant increases in vocalization, lying down, self-directed behavior, and with significant decreases in threatening behavior, exploration, huddling and grooming (Kalin 1983b). Interestingly, no significant behavioral changes were observed following i.c.v. administration (Kalin 1983c and reported anecdotally by Insel, et al. 1984).

The findings above indicate that administration of CRH is capable of inducing behaviors which may be physiologically relevant. It is unclear at present if these effects are mediated directly by CRH, or if they represent indirect consequences of CRH actions on sympathetic and endocrine systems. In view of these possibilities, any investigation purporting to determine behavioral changes following administration of CRH would be strengthened by control studies incorporating interventions that localize its potential mechanism of action (e.g. hypophysectomy).

1-7.f. Clinical Studies Employing CRH

CRH has been used in a limited number of clinical trials. In a double-blind placebo controlled study, Orth, et al. (1983) observed a threshold dose for CRH stimulation of the pituitary-adrenal axis at 0.01 to .03 ug/kg body weight i.v. with a half-maximal dose at 0.3 to 1.0 ug/kg. A dose of 1.0 ug/kg ovine CRF elicited the same magnitude of stimulation of pituitary ACTH release as 10 ug/kg (Orth, et al. 1983). ACTH release was first detectable by 5-15 minutes after injection and peak effects were noted at 30-60 minutes. Interestingly, higher doses of ovine CRH (3.0-30.0 ug/kg) exhibited a second increase in ACTH secretion approximately 4 hours after injection (Orth, et al. 1983, DeBold, et al. 1983, Conaglen, et al. 1984, Chrousos, et

al. 1983, Nakahara, et al. 1983, Tanaka, et al. 1983, Hermus, et al. 1984, Copinschi, et al. 1983). This second and prolonged release is believed due to increased synthesis of POMC which has been noted following longer periods of CRH stimulation in vitro (Bruhn, et al. 1984).

The responsiveness of the pituitary-adrenal axis to CRH is inversely related to basal activity of the axis immediately before injection. For example, an inverse correlation was observed between peak ACTH responses and basal ACTH and cortisol levels (Tanaka, et al. 1983, Hermus, et al. 1984). Not surprisingly, dexamethasone pretreatment blocked responsiveness of the pituitary-adrenal axis to subsequent administration of CRH (Copinschi, et al. 1983). Thus, endocrine status at the time of administration modulates the release of pituitary ACTH and B-END evoked by CRH.

CRH does not appear to stimulate release of other hormones when given to humans. Following administration of up to 500 ug CRH, no changes were observed in plasma levels of GH, LH, FSH, TSH, PRL, AVP, renin, insulin, cholecystokinin, gastrin, Met-ENK, glucagon or catecholamines (Orth, et al. 1983, Conaglen, et al. 1984, Chrousos, et al. 1983, Nakahara, et al. 1983, Copinschi, et al. 1983, Lytras, et al. 1984). Although Hermus, et al. (1984) reported that administration of 200 ug CRH to 10 subjects did produce significant decreases in TSH and PRL,

these observations are not supported by the findings of many others.

Three clinical trials have observed increases in plasma levels of aldosterone (Conaglen, et al. 1984, Nakahara, et al. 1983, Hermus, et al. 1984). This effect may be due directly to CRH or may be due to the known stimulatory effects of POMC related peptides on aldosterone release (Gullner, et al. 1983, Washburn, et al. 1982, Rabinowe, et al. 1985, Matsuoka, et al. 1983, 1984).

Additional changes which may be observed following peripheral administration of high doses of CRH include flushing, dyspnea, hypotension and tachycardia; although the frequency of these has been highly variable. No changes have been observed either in blood chemistries or urinalysis (Orth, et al. 1983, DeBold, et al. 1983). A very rare side effect noted was patient report of anxiety (Orth, et al. 1983, Conaglen, et al. 1984, Tanaka, et al. 1983). On the basis of these observations, risks associated with CRH appear minimal. Nonetheless, clinical trials using this agent should provide assessment of side effect liability.

In conclusion, several lines of evidence support the contention that Vale's 41 amino acid peptide is CRH. It is a potent stimulant of the pituitary-adrenal axis and exhibits other physiologic and behavioral effects which are consistent with its role as a central neurotransmitter. Together, these facts indicate that synthetic CRH is a

useful tool for determining the physiologic functions due to pituitary B-END.

1-8. Rationale For Research Project

The availability of pure synthetic CRH permits a direct examination of the hypothesis that pituitary B-END normally mediates analgesia. In contrast to utilization of stressful stimuli (i.e., SIA), administration of CRH represents a selective intervention due to its reproducability, potency and specificity for stimulating the pituitary-adrenal axis. CRH does not bind to opiate receptors (Dave, et al. 1985b) and thus does not mimic activation of endogenous opioids. In addition, administration of CRH bypasses CNS mechanisms involved in regulating corticotroph secretion. Accordingly, utilization of CRH to selectively stimulate corticotroph release of B-END is a logical strategy for determining the functional significance of blood-borne B-End in mediating adaptive responses to pain and stress.

The physiological relevance of the research project lies in the fact that the potential role of pituitary B-END in behavioral nociception is evaluated directly following the selective stimulation of pituitary corticotroph secretion by CRH. In other words, the primary question of the proposal is addressed by examining the effects of CRH administration. The absence of antinociception following

CRH leads to a rejection of the hypothesis that blood-borne B-END mediates analgesia. However, the presence of CRH produced antinociception supports the primary hypothesis and raises questions which more specifically pertain to the mechanism of CRH action. The most likely possibility is that CRH-induced anti-nociception is due to release of pituitary B-END. However, alternative mechanisms exist including release of pituitary ACTH, release of B-END or other opioid peptides by CNS neurons or direct effects of CRH on peripheral and/or central neurons which transmit nociceptive information.

The hypothesis that modulation of pituitary-adrenal axis activity produces differences in perceived pain was tested in patients undergoing surgical removal of impacted third molars. The clinical studies examined the following questions:

1. Is the oral surgery model appropriate to evaluate endocrine correlates of pain and stress?
2. Does stimulation of the pituitary-adrenal axis result in decreased post-operative pain?
   - tested with CRH
3. Does inhibition of the pituitary-adrenal axis result in increased post-operative pain?
   - tested with dexamethasone

The hypothesis that the release of pituitary B-END mediates CRH-induced antinociception has been tested in the rat hot plate model by examining the following questions:

1. Is CRH-induced antinociception mediated by secretion of an endogenous opioid substance?
   - tested with naltrexone
2. Is CRH-induced antinociception dependent on pituitary activity?
   - tested with hypophysectomized rats
3. Is CRH-induced antinociception due to secretory activity of the pituitary corticotroph?
   - tested with dexamethasone pre-treatment
4. Is CRH-induced antinociception due to circulating immunoreactive B-END?
   - tested with passive immunization with anti-endorphin antiserum

Thus, studies in both humans and rats were conducted. The clinical experiments directly tested whether CRH produces analgesia. Extending these findings, the animal experiments tested whether pituitary B-END mediates CRH-induced antinociception. Together, these parallel studies examined the relationship between pituitary B-END and nociception in both humans and animals.

CHAPTER 2

METHODS

2-1. Clinical Studies

2-1.a. Bioassay Sensitivity For Analgesics Using The Oral Surgery Model

In order to appropriately test the hypothesis that pituitary B-End possesses analgesic activity, a clinical model with documented sensitivity to analgesic drugs must be employed. The oral surgery model ably fulfills this criterion. Numerous clinical trials conducted under randomized double-blind conditions have demonstrated differences between analgesic drugs and placebo, as well as dose related differences in analgesic drugs (reviewed by Troullos, et al. 1986, Cooper 1983). Utilization of normal healthy patients not taking concurrent medications, maximizes the generalizability of the results. Accordingly, drug interactions are minimized in this model, with the minimal requirement consisting of adequate local anesthesia during surgery. The present study further demonstrates that the oral surgery model constitutes a

physiologically relevant approach for investigating the relationship that exits between surgical stress, post-operative pain and the secretion of pituitary B-END in humans.

2-1.b. Patients

All patients in the clinical trials were healthy male or female volunteers with indications for removal of impacted third molars. Subjects free of systemic disease, and not taking concurrent medications, signed an institutionally approved informed consent form which allowed them to withdraw from the study at any time. The average age of the 110 patients who participated in the three clinical studies was 22.2 $\pm$ 0.4 years with 46 females and 64 males.

Patients were initially screened following receipt of a letter of referral indicating a clinical need for third molar removal. The nature of the clinical experimental procedures, the drugs, the purpose of the study and the risks and inconveniences associated with both the extractions and the experimental protocol were explained to the patients orally and in the informed consent document. A complete medical history was elicited from the patient and transcribed for the medical record. An oral and radiographic examination was then performed to confirm the indication for the extraction and to determine

that the candidate satisfied the inclusion criteria for the study. Inclusion criteria included American Society of Anesthesiology class I health status, and bilaterally symmetrical impactions (CRH study only). Exclusion criteria included a history of serious illness, endocrine dysfunction, or drug abuse. Subjects whose past medical history indicated that they may have been at possible risk due to the surgical or experimental procedures were evaluated by a National Institute of Dental Research (NIDR) staff physician prior to surgery. All patients on the CRH study underwent routine physical exams.

2-1.c. Surgery and Drugs

Patients underwent standardized oral surgery at the Pain Research Clinic of the NIDR under institutionally approved protocols.

All drugs were stored, prepared and randomized by the Investigational Pharmacy of the Clinical Center, NIH. The human CRH was synthesized and purified commercially (Bachem, Inc., Torrance, CA). Purity and potency of the dosage form was confirmed in the Analytical Section of the Investigational Pharmacy. Sterility and pyrogen testing was performed by the Bureau of Biologics according to U.S.P. specifications. CRH was used under a Food and Drug Administration Investigational New Drug Application #23,360. Dexamethasone, naloxone, fentanyl, diazepam and

local anesthetics were purchased from commercial sources. The pharmacy prepared 2% mepivacaine from commercially available 3% mepivacaine. Except for local anesthetics, all drugs were administered intravenously double-blind with the code maintained by the Investigational Pharmacy.

2-1.d. Quantitation of Pain, Anxiety and Other Measures

Perceived levels of pain and anxiety were measured with several scales. These scales are presented in Appendix 1. Patient reports of pain were quantitated with a visual analog scale (VAS). The VAS is a 10 cm line bounded on the left with the words "no pain" and on the right with "pain as much as it could be." The patient is instructed to place a mark on the scale proportional to their pain. Although simplistic, the VAS is a sensitive measure and satisfies at least minimal assumptions for parametric statistical analysis (Huskisson 1983). Equally important, the VAS has been demonstrated to detect analgesic effects of active drugs, as compared to placebos, in the oral surgery model (Troullos, et al. 1986, Cooper 1983, Dionne, et al. 1983).

Two components of the pain experience, the sensory intensity and unpleasantness of pain, were also measured. Verbal descriptor scales were employed in the first clinical trial (2-1.f) to provide a second measure for pain as well as to measure separately the sensory intensity and

unpleasantness components of pain (Gracely 1979). The CRH (2-1.g) and dexamethasone (2-1.h) clinical studies utilized newly developed and validated VAS scales to measure separately the sensory intensity and unpleasantness components of pain (Price, et al. 1983).

Patient reports of anxiety were also measured. In the first clinical study (2-1.f), where pre-operative anxiety constituted a significant component of stress, samples were collected using both a VAS and a verbal descriptor scale (Spielberg's State-Trait Anxiety Index, STAI). The second (2-1.g) and third (2-1.h) clinical trials concentrated on post-operative time points. Since anxiety is low at this time (discussed in 3-1.a), only one scale for anxiety (VAS) was used.

The time course for the offset of local anesthesia as well as the surgeons rating of surgical trauma were also measured. Patient report of local anesthesia was measured on a category scale (numb, tingling, normal). The surgeon's rating of trauma was also measured on a category scale, reproduced in the appendix. These scales are necessary to determine if group differences in duration of local anesthesia or amount of surgical trauma underlie differences in patient report of pain. The absence of significant differences between groups for these parameters is a necessary prerequisite prior to interpretation of differences in pain as due to differences in drug treatment.

Since administration of CRH has been associated with transient alterations in cardiovascular parameters (Orth, et al. 1983, DeBold, et al. 1983), patients participating in the CRH study (2-1.g) were monitored throughout the trial. Blood pressure was determined with a sphygmomanometer, while heart rate was taken from a lead II electrocardiogram (Life Pak 6 monitor, Physio Control Inc., Redmond, WA). Oral temperature was measured by an IVAC 821A (IVAC Inc., San Diego, Ca). Facial flushing was assessed by the investigator on a +/- scale based on the patient's facial tone prior to drug administration.

2-1.e. Determination of Plasma Hormone Levels

Plasma levels of iB-END were determined as previously described (Mueller 1980, Hargreaves, et al. 1983). For purposes of monitoring peptide recovery, plasma samples (6 ml) were spiked with a trace amount of internal standard ^{125}I-B-END and then loaded onto octadecasilysilica resin columns (C-18 Sep Pak Columns, Waters Inc., Milford, MA), washed with 3 ml 0.05% trifluoroacetic acid, and eluted with 3 ml 50% acetonitrile containing 0.05% trifluoroacetic acid. Samples were concentrated by rotary evaporation and lyophilization.

Following resuspension in assay buffer, the samples were assayed for iB-END by radioimmunoassay (RIA) (Mueller 1980). The RIA employs a polyclonal antisera raised in our

laboratory which can detect less than 1 fm camel B-END_{1-31} standard (Peninsula Laboratories, San Carlos, CA). The antibody recognizes B-END positions 17-26 and thus detects B-LPH equally, but does not recognize alpha endorphin, alpha MSH, the enkephalins, CRH, morphine, or hormones of the gastrointestinal tract.

Plasma levels of immunoreactive cortisol (i-cortisol) were measured using a commercially available radioimmunoassay kit (Travenol/Genentech Inc. Boston, MA). Circulating levels of epinephrine and norepinephrine were quantitated by HPLC followed by electrochemical detection in the laboratory of Dr. David Goldstein, NHLBI according to published methods (Goldstein, et al. 1981b).

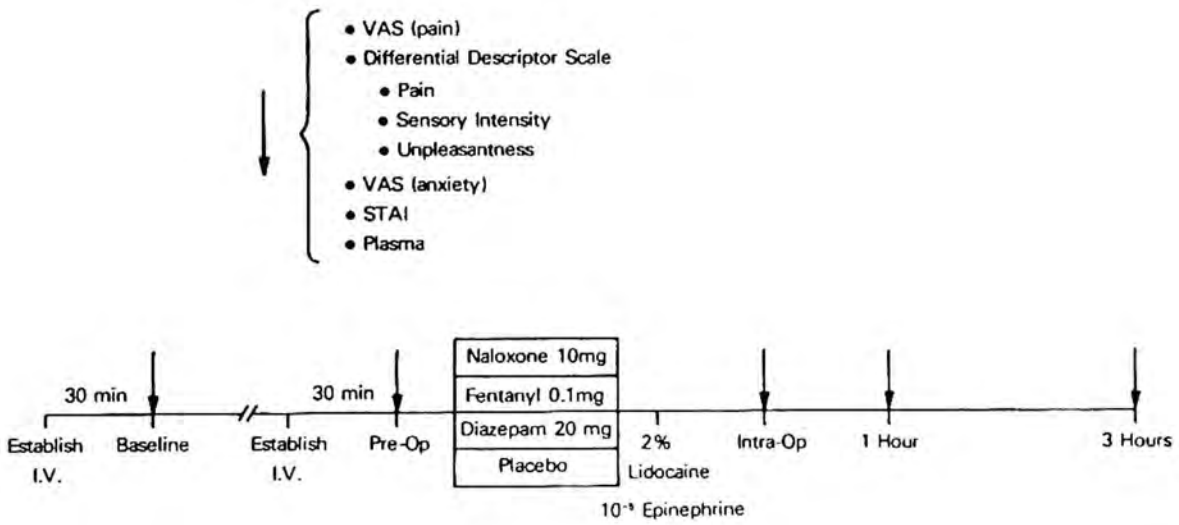
2-1.f. Endorphin Drug Study

Forty-eight informed volunteers who were accepted at the Pain Research Clinic of the NIDR with at least two impacted third molars were the subjects of this study. All drugs were administered double-blind.

The experimental design is presented in Figure 2. Patients reported to the clinic at least one week prior to surgery for a baseline sample. An intravenous infusion was established in a vein in the antecubital fossa and, following a 30 minute recovery period, a blood sample was drawn and questionnaires administered. Blood samples were collected with EDTA (1.4 mg/ml) and centrifuged. The

Figure 2. Experimental design and sampling schedule for the endorphin drug study. Forty-eight patients were randomly allocated to 4 treatment groups and were administered in a double-blind fashion naloxone 10 mg, fentanyl 0.1 mg, diazepam 0.3 mg/kg (average dose 20 mg) or placebo. Samples were collected as indicated. Surgery consisted of removal of 2-4 impacted third molars.

PHARMACOLOGICAL MODIFICATION OF PITUITARY BETA-ENDORPHIN RELEASE



resulting plasma was frozen over dry ice and stored at -70 C. Questionnaires for perceived levels of pain and anxiety included VAS scales for pain and anxiety and the STAI. Verbal descriptor scales were employed to provide a second measure for pain, as well as to measure separately the sensory intensity and unpleasantness components of pain.

On the day of surgery, an intravenous infusion was reestablished with a 30 minute recovery prior to the pre-operative sample. Patients were randomly assigned to one of 4 treatment groups and given either intravenous saline placebo, naloxone (10 mg), fentanyl (0.1 mg) or diazepam (0.3 mg/kg) five minutes before start of surgery. All groups then received 2% lidocaine containing 1:100,000 epinephrine for local anesthesia. Intra-operative blood was withdrawn during surgery, with at least one tooth extracted, while questionnaires were given immediately after surgery was completed. Additional blood samples and questionnaires were taken 1 and 3 hours post-operatively. Plasma levels of iB-END and catecholamines were determined as described.

2-1.g. CRH Study

The CRH trial was a randomized double-blind, counter-balanced cross-over design using 14 male patients each completing two separate surgeries. The cross-over design was selected to minimize variability and to maximize

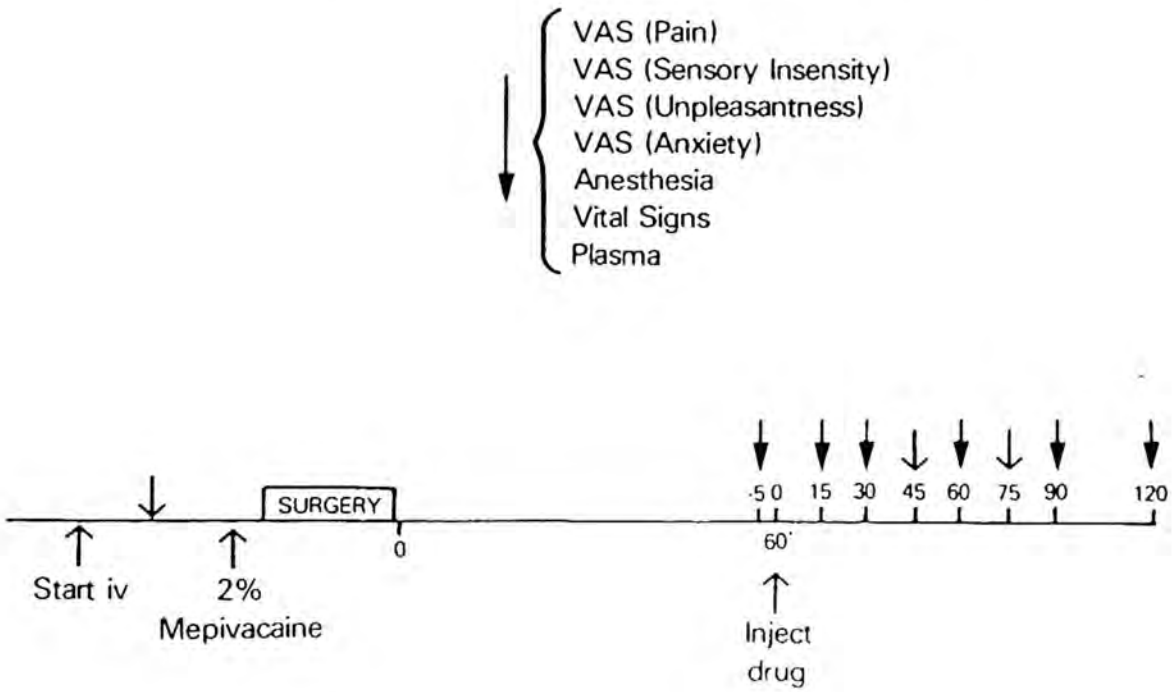
the ability of the study to detect a treatment effect (i.e., CRH-induced analgesia). Each patient underwent a complete medical history, physical exam and laboratory tests prior to enrollment. Patients with bilaterally impacted third molars in need of removal served as subjects at two separate appointments. All surgeries were started in the afternoon to minimize endogenous CRH activity (it is impractical to conduct surgeries in the late evening when time would be optimal).

At each appointment, an upper and lower third molar on the same side were extracted under local anesthesia consisting of 2% mepivacaine. Figure 3 provides a summary of the sampling schedule. At 60 minutes following surgery, subjects were administered (i.v.) 1.0 ug/kg CRH or placebo on a random double-blind basis while in the supine position. Half of the patients received CRH on the first appointment and the other half were administered placebo first. Questionnaires were administered (see Appendix 1) and blood samples collected immediately prior to and at 15, 30, 45, 60, 75, 90 and 120 following drug administration (questionnaires only at 45 and 75 minutes). Vital signs were monitored throughout the test. At a second appointment approximately 2 weeks later, subjects had the remaining two third molars extracted and received the alternative treatment.

The primary hypothesis tested for CRH analgesia through evaluation of pain reports using scales previously

Figure 3. Experimental design and sampling schedule for the clinical CRH study. Fourteen patients participated in this double-blind cross-over study in which an impacted maxillary and mandibular third molar were surgically removed at each of two appointments. At 60 minutes following surgery, patients were administered either CRH 1 ug/kg or placebo and the alternative treatment was given after the second surgery. Samples were collected as indicated.

EFFECTS OF CRH ON ACUTE POST-OPERATIVE PAIN



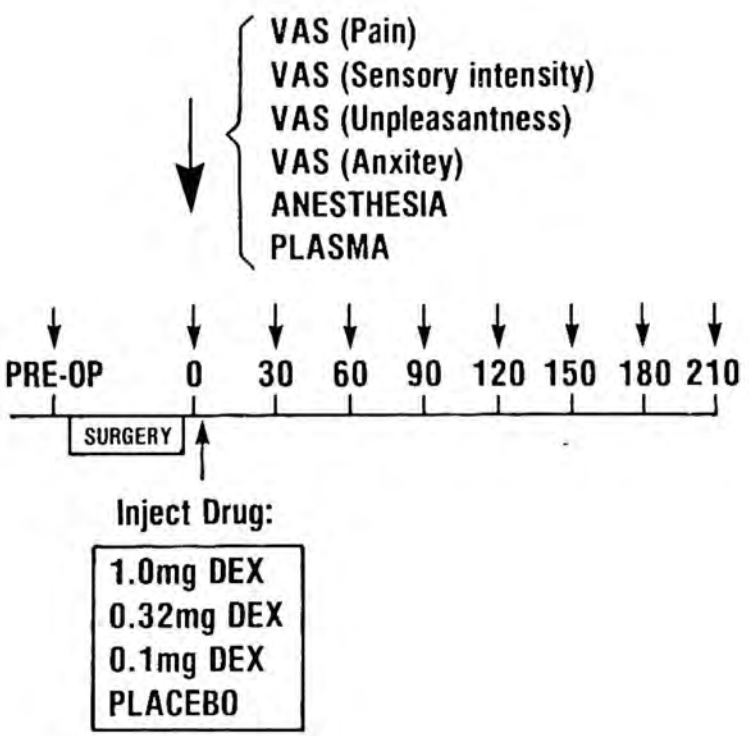
demonstrated to be sensitive for detecting analgesic effects of drugs (see 2-1.d). These scales are 1) VAS for pain; 2) VAS for unpleasantness; and 3) VAS for sensory intensity. Other statistical analysis evaluated equality of the groups by testing whether differences in pain report following placebo and CRH were due to differences in surgical trauma (assessed by the oral surgeon) or level of anesthesia (patient self-report). It was unlikely that significant differences in either trauma or anesthesia would occur, since patients were screened for symmetrical third molar impactions. Additional analyses tested for side effect liability, including CRH-induced changes in perceived anxiety, based on anecdotal reports (Orth, et al. 1983, Conaglen, et al. 1984, Tanaka, et al. 1983), and gross cardiovascular effects. Bioactivity of CRH in post-operative patients was evaluated by determining its effect on plasma levels of iB-End and i-cortisol.

2-1.h. Dexamethasone Study

The third clinical trial employed a double-blind randomized dose response design for determining the effects of placebo or 0.1 mg, 0.32 mg or 1.0 mg dexamethasone (i.v.) administered post-operatively to 48 patients. Patients had an upper and lower third molar extracted under local anesthesia consisting of 2% mepivacaine. An overview of the sampling schedule is presented in Figure 4. At 10

Figure 4. Experimental design and sampling schedule for the clinical dexamethasone study. Forty-eight patients were randomly allocated to 4 treatment groups and at 10 minutes following surgery were administered either placebo, 0.10 mg, 0.32 mg or 1.0 mg of dexamethasone under double-blind conditions. The samples were collected as shown.

EFFECTS OF DEXAMETHASONE ON POST-OPERATIVE PAIN



minutes after surgery, patients were administered placebo or 0.1 mg, 0.32mg, or 1.0 mg dexamethasone on a random basis double-blind. Questionnaires were administered and blood samples collected prior to surgery, 5 minutes after surgery, and at 30, 60, 90, 120, 150, 180 and 210 minutes (questionnaires only at 210 minutes) after drug administration. Blood samples were processed and assayed as described (2-1.e).

Since the primary hypothesis was to determine if suppression of the pituitary-adrenal axis resulted in greater post-operative pain, a confound could occur if a predominant anti-inflammatory effect was produced by the dose of dexamethasone. A dose-response design was selected since a review of the literature indicated that doses of dexamethasone required to suppress the pituitary-adrenal axis are much lower than that required for clinically detectable anti-inflammatory effects (Williamson, et al. 1980, Haynes and Larner 1983). Initial pilot studies determined the dose range for the study.

In addition to testing for drug effects on pain, other statistical analyses evaluated equality of the groups by testing for differences in surgical trauma or self report of anesthesia. Analysis of the effect of dexamethasone suppression of the pituitary-adrenal axis was made following assay of plasma levels of iB-End.

Thus, the CRH and dexamethasone clinical studies together involved 76 patient-surgeries. Each study

determined the effect of either stimulation or suppression of the pituitary-adrenal axis on post-operative pain. While the clinical studies required the major degree of effort in the research project, parallel studies in animals were required to test alternative hypotheses regarding the mechanism of CRH induced antinociception (see 1-8).

2-2. Animal Studies

2-2.a. Bioassay Sensitivity For Antinociceptive Drugs Using The Hot Plate Model

The effectiveness of 50 C versus 55 C hot plate models in screening for analgesic drugs has been widely reported (O'Callaghan and Holtzman 1975, Ankier 1974, Taber 1974, Evans 1964, Keith 1960). In general, the lower temperature thermal plate model (i.e. 49.5 - 50.0 C) detects lower doses of morphine as well as weaker analgesic drugs such as pentazocine, levallorphin or nalorphine (Ankier 1974, O'Callaghan and Holtzman 1975). For example, Ankier (1974) has demonstrated that the ED_{50} for morphine using a 50 C thermal plate, is approximately 1/3 of the ED_{50} dose required for the 55 C plate. Importantly, the 50 C thermal plate model predicts analgesic activity in humans and provides similar relative potency data (Ankier 1974). Thus, the 50 C thermal plate model detects smaller doses of strong analgesic drugs (e.g. morphine) as well as drugs

possessing moderate analgesic efficacy; effects which may be missed if evaluated by a 55 C thermal plate.

This issue is relevant since preliminary studies (reported in the research proposal) demonstrated a significant CRH-induced antinociception, when compared to vehicle, using a 50 C thermal plate (45.0 $\pm$ 4.0 seconds vs. 32.1 $\pm$ 3.2 seconds, $p < 0.05$) but not a 55 C plate (13.5 $\pm$ 2.7 seconds vs 11.5 $\pm$ 1.3 seconds, non-significant (ns)). Hence, the 50 C temperature was selected for the studies reported here.

The dependent measure employed in the rat hot plate studies was the latency between exposure to the hot plate and elicitation of a hind paw lick. Rats rarely (<5%) jumped under conditions of the 50 C plate. The hind paw lick has been used as a behavioral endpoint in several previous studies (Dennis and Melzack 1980, Jensen and Yaksh 1985, Woolfe and MacDonald 1944, Schmaus and Yaksh 1984, Yeung and Rudy 1980a,b, Yaksh and Rudy 1976). As an endpoint, the hind paw lick avoids potential false-positives such as grooming (a fore-paw mediated behavior). Thus, fore-paw lick, if included as an endpoint in the dependent measure, can constitute a false-positive measure of behavior nociception, due to the potential confound of grooming behavior. Since CRH, at least when administered centrally, can invoke grooming behavior (Koob and Bloom 1985, Veldhuis and DeWied 1984, Morley and Levine 1982a,

Britton, et al. 1982,1984), the dependent measure was restricted to hind paw lick as the endpoint.

Although a 50 C thermal plate may suggest innocuous stimuli, it is important to recall that the 50 C surface exceeds the 45 C cutaneous pain threshold observed in numerous studies (reviewed in Willis 1985, Dubner and Bennett 1983) and is clearly capable of evoking a pain response (Hargreaves, personal observation). Although pain is perceived, tissue damage is not likely to occur under the conditions employed here. The hind paw lick is elicited at the temperature of 50 C, but not at temperatures below the pain threshold. In a preliminary study of 17 rats, a hind paw lick at 43 C was not observed over a 180 second observation period; when the temperature was raised to 50 C the hind paw lick latency was 45.2 $\pm$ 3.4 seconds ($p < .01$). Thus, as a dependent measure for behavioral nociception, the hind paw lick latency avoids potential false-positives and is elicited at temperatures in the noxious range.

2-2.b. Experimental Designs

A sequential series of animal studies has been designed to examine the role of pituitary B-END in antinociception.

First, the optimal time and dose for CRH to produce antinociception was determined. This experiment

established the conditions for subseqeunt studies testing the pituitary B-END hypothesis. The antinociceptive properties of CRH were characterized by comparison of paw lick latencies to a vehicle and to a positive control. For this purpose, dose-response curves for CRH (6.3, 12.6, 25.2 nmoles/kg) and morphine sulfate (7.5, 15.0, 30.0 umoles/kg = 2.5, 5.0, 10.0 mg/kg) were determined.

The second experiment evaluated the effects of opiate receptor blockade on CRH-induced behavioral nociception. Accordingly, this study determined the effects of pretreatment with naltrexone or naltrexone methylbromide (a quarternary analog) on subsequent CRH-induced antinociception. It is important to note that CRH does not bind to opiate receptors (Dave, et al. 1985b). Thus naltrexone blockade of CRH-induced antinociception implies that the observed effect is due to CRH-induced secretion of an endogenous opioid. Naltrexone was selected in favor of naloxone, since the former has a much longer half-life (Smith 1979). A dose of 1 mg/kg was selected to block primarily mu opiate receptors, while 10 mg/kg was employed to block mu as well as other opiate receptors. A dose of 10 mg/kg of the quaternary analog of naltrexone (naltrexone methylbromide) was employed due to differences in relative potency as compared to the parent, tertiary analog (Brown and Goldberg 1985). The quaternary analog of naltrexone possesses a positive charge, and thus poorly penetrates the BBB (Brown and Goldberg 1985). Blockade of

analgesia by the quaternary naltrexone may be interpreted to indicate a peripheral mechanism in the development of the observed analgesia. Naltrexone was obtained from Dr. V. Nicholson, Endo/Dupont Labs., Glenolden, PA. Naltrexone methylbromide (MRZ 2663) was obtained from Ms. H. Reides-Esche, Boehringer-Ingelheim LTD, Ridgefield, CT.

The third study evaluated the necessity of an intact pituitary gland for CRH-induced antinociception. This study blocked activation of the pituitary-adrenal axis by hypophysectomy. Rats underwent a complete parapharyngeal hypophysectomy or underwent a similar surgical procedure leaving an intact pituitary ("sham" group) 3 weeks prior to the study. Surgeries were performed at Hilltop Farms, Scottsdale, PA. Due to the diverse metabolic effects of glucocorticoids and their effects on the BBB (Long and Holaday 1985), hypophysectomized rats received glucocorticoid replacement therapy (corticosterone, 0.5 mg i.p. b.i.d., Sigma Chemicals, St. Louis, MO). Sham rats received i.p. injections of saline. Completeness of hypophysectomy was determined by the lack of change in plasma levels of iB-End following challenge with CRH.

The fourth experiment determined the effects of blockade of pituitary corticotroph activity by dexamethasone pretreatment, as compared to vehicle, on CRH-induced antinociception. The dose of dexamethasone (0.5 mg/kg i.p., 2 hours prior to testing) had been previously

determined to block pituitary corticotroph responsiveness to stress challenges (Mueller, et al. 1985). Note that both hypophysectomy and dexamethasone possess diverse physiologic effects. Yet they share a common impact on blocking pituitary corticotroph activity. Thus both interventions must block CRH antinociception to make an interpretation about pituitary corticotroph participation.

The next study evaluated the effects of passive immunization with anti B-END antiserum on CRH-induced antinociception. Of the three possible mechanisms by which pituitary B-End could be antinociceptive (see 1-5), two require access to the systemic circulation (e.g. crossing the BBB and activating peripheral opiate receptors). Thus, passive immunization by intravenous injection of anti-B-End as compared to hyperimmune serum (for control) may block development of CRH-induced antinociception. The anti-endorphin antisera was C-55, the same antisera employed in the endorphin RIA. The control hyperimmune antisera was raised by injecting rabbits with complete Freund's adjuvant with bovine thyroglobulin only. Rats were passively immunized i.v. with 250 ul of either anti-endorphin antiserum or hyperimmune antiserum.

2-2.c. Experimental Schedule

The initial rat experiments optimized the CRH dose (25.2 nmoles/kg) and experimental schedule. Ovine CRH

(Peninsula Laboratories, San Carlos, Ca) was dissolved at 120 ug/ml in a degassed vehicle consisting of 10 mg/ml bovine serum albumin fraction V (Sigma Chemical, St. Louis, MO), 1 mg/ml L-ascorbic acid (Fisher Scientific, Fair Lawn, NJ) and 9 mg/ml NaCl (Pioneer Chemical Co., New York, NY). Aliquots of CRH or vehicle were randomly assigned a drug code and immediately frozen. In studies utilizing pre-treatment with another drug (e.g. naltrexone, dexamethasone, etc.), the active drug or appropriate vehicle was dispensed in numbered vials and stored frozen. Aliquots were thawed once only, immediately prior to injection, and all samples were used within 5 days.

Male Sprague-Dawley rats (Hilltop Farms, Scottsdale, PA) weighing 225-300 grams were housed in the Department of Laboratory Animal Medicine, USUHS, for at least one week prior to study with lights on from 0600 - 1800 and food and water ad libitum. Rats were accustomed to daily handling for 3 days prior to and on the day following surgical catheterization.

On the day of surgery, rats were anesthetized with sodium pentobarbital (50 mg/kg, ip) and maintained, when necessary, with ethyl ether. The left external jugular vein was exposed by a ventral incision and catheterized with tygon microbore tubing (.04 x .07 inches, Fisher Scientfic, Fairlawn, NJ) with one end previously stretch-thinned (following immersion into oil at 190 C). The catheter was exteriorized through a dorsal incision in the

neck and remained patent by the addition of heparin (50 ul of 1,000 units/ml i.v., Lypho Med, Chicago, IL). Animals were then housed individually, with additional heparin administered i.v. 24 hours later.

On the test day, 48 hours after surgery, baseline nociceptive reactions to heat were assessed using a 50 C thermal plate with a 19 x 23 cm plastic cylinder (Technilab Inst. Co., Model 475, Pequannock, NJ). The cut-off was 90 seconds. Drugs were administered i.v. or i.p. according to the experimental design (see results). In all studies the dependent measure, hind paw lick latency, was assessed by an observer blind as to treatment allocation. The studies were conducted using complete block repeated measures designs, with samples collected at a baseline session one hour prior to drug administration, and at 15, 30, 45 and 60 minutes after drug administration. Following completion of the study, at 75 minutes after drug injection, animals were decapitated with trunk blood collected in plastic tubes containing 600 ul of 10% EDTA and 0.5 mg% bacitracin. Aliquots of plasma were assayed directly (without sep pak extraction) for iB-END by RIA as described in section 2-1.e.

2-3. Statistical Analysis

All dependent measures, from both clinical and animal studies, were collected under double-blind

conditions. The data were analyzed by ANOVA (Winer 1975), followed by Duncan's new multiple range test (Duncan 1955) using a computer program (Biomedical Data Processing (BMDP), Dixon, et al. 1983) available at the NIDR Computer Center.

CHAPTER 3

RESULTS

3-1. Clinical Studies

3-1.a. Endorphin Drug Study

This study determined the effects of prototype drugs on modifying plasma iB-END responses to pre-operative anxiety, surgical stress and the onset of acute post-operative pain in the oral surgery model. The experimental design and sampling schedule are presented in Figure 2.

Because at the pre-operative sample the patients had yet to be segregated into treatment allocations, the interaction of anticipatory anxiety and plasma iB-END level was analyzed for the group as a whole. Presurgical anxiety levels measured by the STAI were significantly elevated from the baseline period 1 week before surgery (31.2 ± 1.4) to the pre-operative period (40.0 ± 2.4; $p < 0.01$). The VAS for anxiety also exhibited a significant increase, from 5.4 ± 1.6 to 23.6 ± 2.2 ($p < 0.01$). Despite the significant increase in anticipatory anxiety, plasma levels

of iB-END did not change from baseline (24.6 ± 1.1 pg/ml) to the pre-operative sample (24.9 ± 2.2 pg/ml).

After the start of the surgical procedure, plasma levels of iB-END varied with the drug regimen (Fig. 5). The stress of surgery in patients who received placebo resulted in a significant increase in plasma levels of iB-END from the pre-operative (25.6 ± 4.6 pg/ml) to the intra-operative (42.6 ± 9.3 pg.ml) period. An increase in plasma iB-END level from the pre-operative sample (23.6 ± 4.9 pg/ml) to the intra-operative sample (82.2 ± 24.8 pg/ml) also occurred after naloxone administration. Intra-operative levels of iB-END were greater for the naloxone group as compared to the placebo group. Intra-operative B-END levels for both the fentanyl (19.6 ± 6.7 pg/ml) and diazepam (24.8 ± 2.9 pg/ml) groups were less than levels in patients who received pretreatment with either placebo or naloxone. Plasma iB-END levels for all groups fell to near baseline levels at 1 hour after surgery. In all groups, plasma iB-END levels increased from 1 hour to the 3 hour post-operative sample, coinciding with the onset of acute post-operative pain.

Changes in circulating levels of norepinephrine over time also varied with the drug regimen (Table 1). During the stress of surgery, patients who received placebo had increased plasma norepinephrine levels which then decreased 1 hour after surgery and increased again by the 3 hour post-operative sample. Patients receiving naloxone

Figure 5. Effects of naloxone (NAL), fentanyl (FEN), diazepam (DZP) and placebo (PLBO) on circulating levels of iB-END. Drugs were administered immediately prior to oral surgery with samples collected as indicated in Figure 2. N = 12/group. * Significantly different from the placebo group ($p < 0.05$).

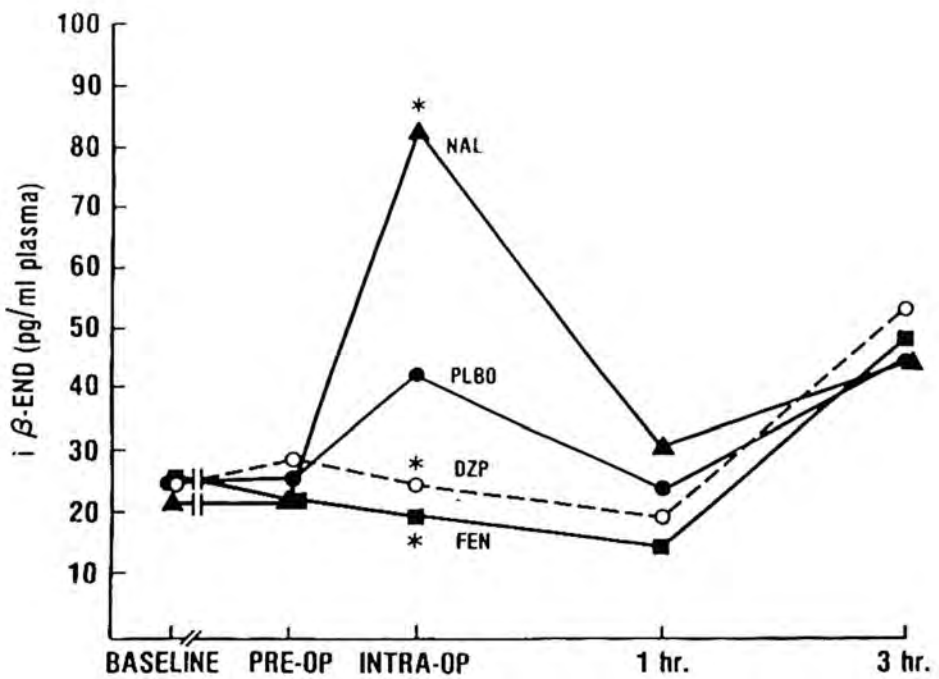

100
90
80
70
60
50
40
30
20
10
i β-END (pg/ml plasma)
NAL
PLBO
DZP
FEN
*
BASELINE
PRE-OP
INTRA-OP
1 hr.
3 hr.

Table I. Effects of placebo, naloxone, fentanyl, and diazepam on plasma norepinephrine levels (pg/ml plasma)

	Baseline	*Preoperative*	*Intraoperative*	*1 hr*	*3 hr*
Placebo	162.7 ± 30.2	196.1 ± 35.0	335.0 ± 68.5*	228.4 ± 60.4†	394.7 ± 107.2†
Naloxone	213.2 ± 31.2	187.6 ± 28.1	220.2 ± 28.5†	195.8 ± 24.7	309.2 ± 57.6*
Fentanyl	251.2 ± 30.4	208.1 ± 36.7	265.4 ± 37.7†	221.0 ± 27.5	401.4 ± 50.7*
Diazepam	169.0 ± 26.4	231.8 ± 32.6	237.2 ± 38.2	250.2 ± 40.0	261.6 ± 26.0

Data are $\bar{X}$ ± SE.
*$P < 0.01$, †$P < 0.05$ compared with previous value.

pretreatment had a smaller, but significant increase in intra-operative norepinephrine levels. This change in norepinephrine levels was less than that in the placebo group. In the fentanyl group, there were increases in plasma levels of norepinephrine in the intra-operative and 3 hours post-operative samples. In contrast, diazepam pretreatment was associated with stable plasma norepinephrine levels throughout the sampling times, with significantly less norepinephrine response to surgery as compared with patients receiving placebo. Plasma levels of epinephrine increased significantly for all groups during surgery and fell to about baseline levels by the 1 and 3 hour post-operative samples (data not shown). However, plasma levels of epinephrine were confounded by the use of epinephrine in the local anesthetic.

Patient reports of pain, as measured by the VAS, also varied with drug treatment (Fig. 6). The naloxone group reported significantly greater levels of intra-operative pain (36.4 $\pm$ 8.2) than the placebo group (13.2 $\pm$ 2.9). Similar enhancement of reported surgical pain by the naloxone group was also observed with the verbal descriptor scales (Fig. 7). Naloxone pretreatment was associated with significantly greater intra-operative pain (8.8 $\pm$ 1.7) as compared to placebo (3.4 $\pm$ 0.8) on the descriptor pain scale. This effect was also seen for the unpleasantness component of pain (9.2 $\pm$ 1.0 vs. 4.2 $\pm$ 1.1) but not for the sensory intensity component. For all the groups, pain

Figure 6. Effects of naloxone (NAL), fentanyl (FEN), diazapam (DZP) and placebo (PLBO) on patient reports of pain as measured by a VAS. The drugs were administered (N = 12/group) prior to oral surgery using the sampling schedule presented in Figure 2. ** Significantly different from the placebo group ($p < 0.01$).

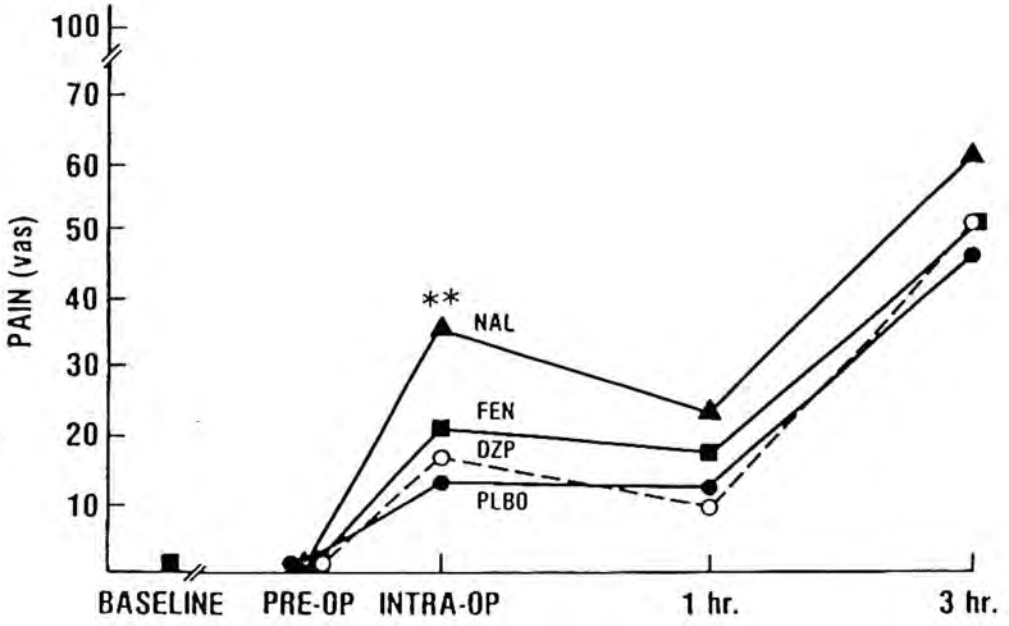
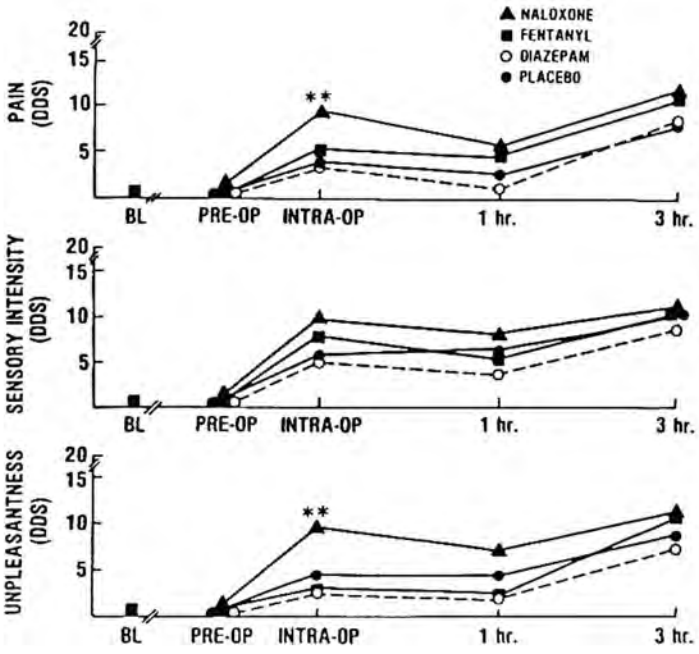

100
70
60
50
40
30
20
10
PAIN (vas)
**
NAL
FEN
DZP
PLBO
BASELINE
PRE-OP
INTRA-OP
1 hr.
3 hr.

Figure 7. Effects of naloxone, fentanyl, diazepam and placebo on patient reports of pain using three verbal descriptor scales, the differential descriptor scales (DDS). The drugs were administered prior to oral surgery as indicated in Figure 2. Top Panel: Patient reports of the magnitude of pain. Middle Panel: Patient reports of the sensory intensity of pain. Bottom Panel: Patient reports of the unpleasantness of pain. ** Significantly different from the placebo group ($p < 0.01$).


▲ NALOXONE
■ FENTANYL
○ DIAZEPAM
● PLACEBO
PAIN (DDS)
SENSORY INTENSITY (DDS)
UNPLEASANTNESS (DDS)
20
15
10
5
**
BL
PRE-OP
INTRA-OP
1 hr.
3 hr.

diminished slightly at 1 hour and increased again at 3 hours with the offset of local anesthesia (Figs. 6,7).

All groups had an increase in anticipatory anxiety from the baseline to pre-operative times as measured by the STAI (Fig. 8). During surgery, patients who received placebo reported greater levels of anxiety (48.0 $\pm$ 2.5) as compared with their pre-operative levels (36.8 $\pm$ 2.7). The naloxone group did not significantly differ in intra-operative anxiety from the placebo group (Fig. 8). In contrast, less intra-operative anxiety was reported by patients given either diazepam (29.3 $\pm$ 1.8) or fentanyl (36.9 $\pm$ 3.1) as compared with patients receiving placebo. Anxiety reports decreased by 1 hour after surgery and remained low at 3 hours (Fig. 8).

Parallel results were observed on the VAS scale for anxiety (Fig. 9). As measured on the VAS scale, the stress of surgery in patients receiving placebo resulted in a significant increase in anxiety from the pre-operative (18.4 $\pm$ 5.0) to the intra-operative (44.2 $\pm$ 8.2) periods. Intra-operative reports of anxiety for patients receiving naloxone (66.9 $\pm$ 17.8) did not significantly differ from those of patients receiving placebo. Intra-operative levels of anxiety for both the diazepam (14.5 $\pm$ 5.8) and fentanyl (20.2 $\pm$ 5.9) groups were less than levels in patients who received placebo.

Patient self-reports of side effects were similar to those usually reported during and after oral surgery

Figure 8. Effects of naloxone (NAL), fentanyl (FEN), diazepam (DZP) and placebo (PLBO) on patient reports of anxiety, as measured using a verbal descriptor scale, the STAI. The drugs (N = 12/group) were given at the dosage and schedule presented in Figure 2. * Signficantly different from placebo ($p < 0.05$). ** Significantly different from placebo ($p < 0.01$).

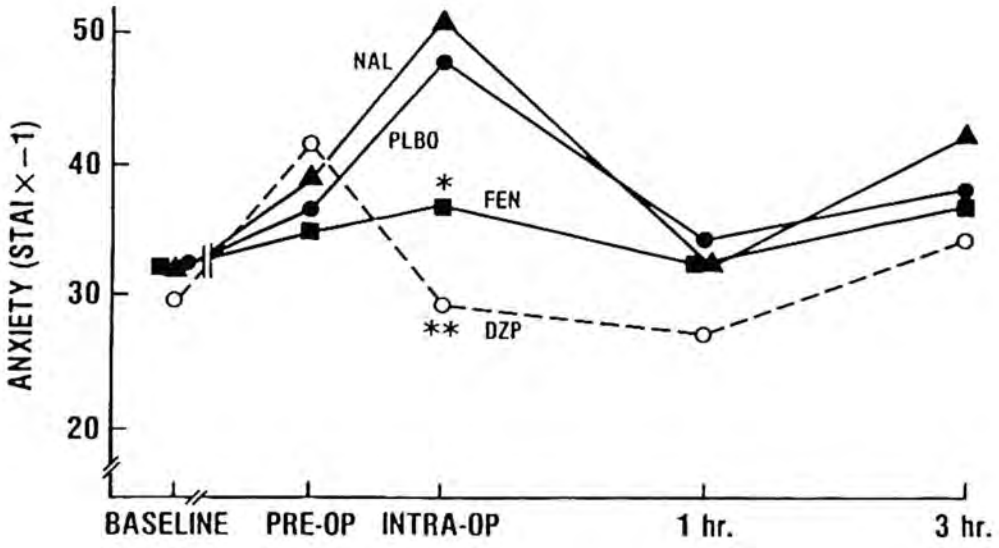
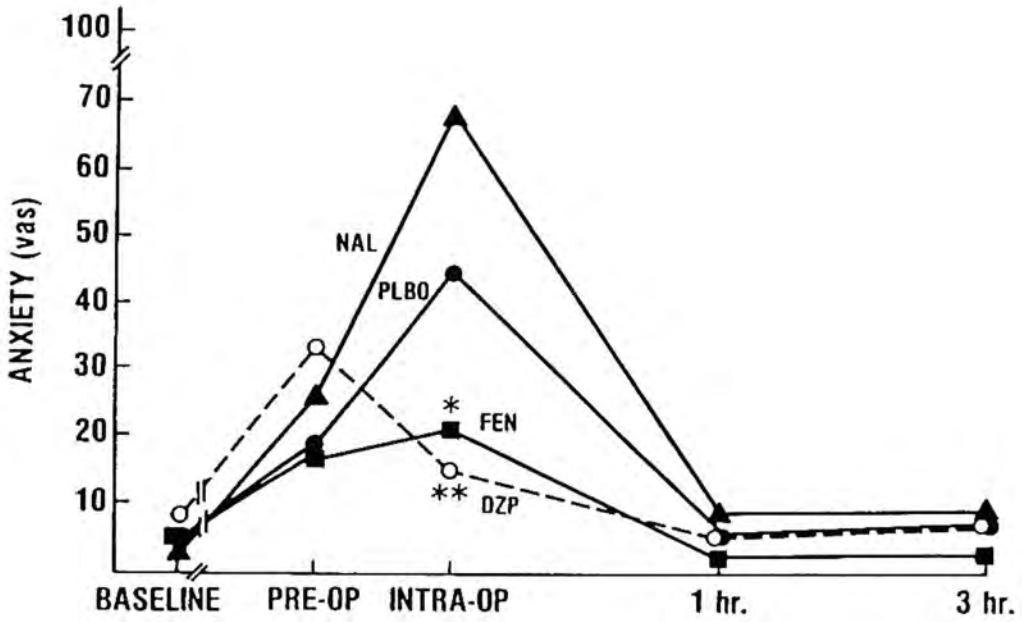

50
40
30
20
ANXIETY (STAI X−1)
NAL
PLBO
FEN
DZP
*
**
BASELINE
PRE-OP
INTRA-OP
1 hr.
3 hr.

Figure 9. Effects of naloxone (NAL), fentanyl (FEN), diazepam (DZP) and placebo (PLBO) on patient reports of anxiety as measured with a VAS. The drugs (N = 12/group) were administered using the dosage and schedule depicted in Figure 2. * Significantly different from placebo ($p < 0.05$). ** Significantly different from placebo ($p < 0.01$).


100
70
60
50
40
30
20
10
ANXIETY (vas)
NAL
PLBO
FEN
DZP
*
**
BASELINE
PRE-OP
INTRA-OP
1 hr.
3 hr.

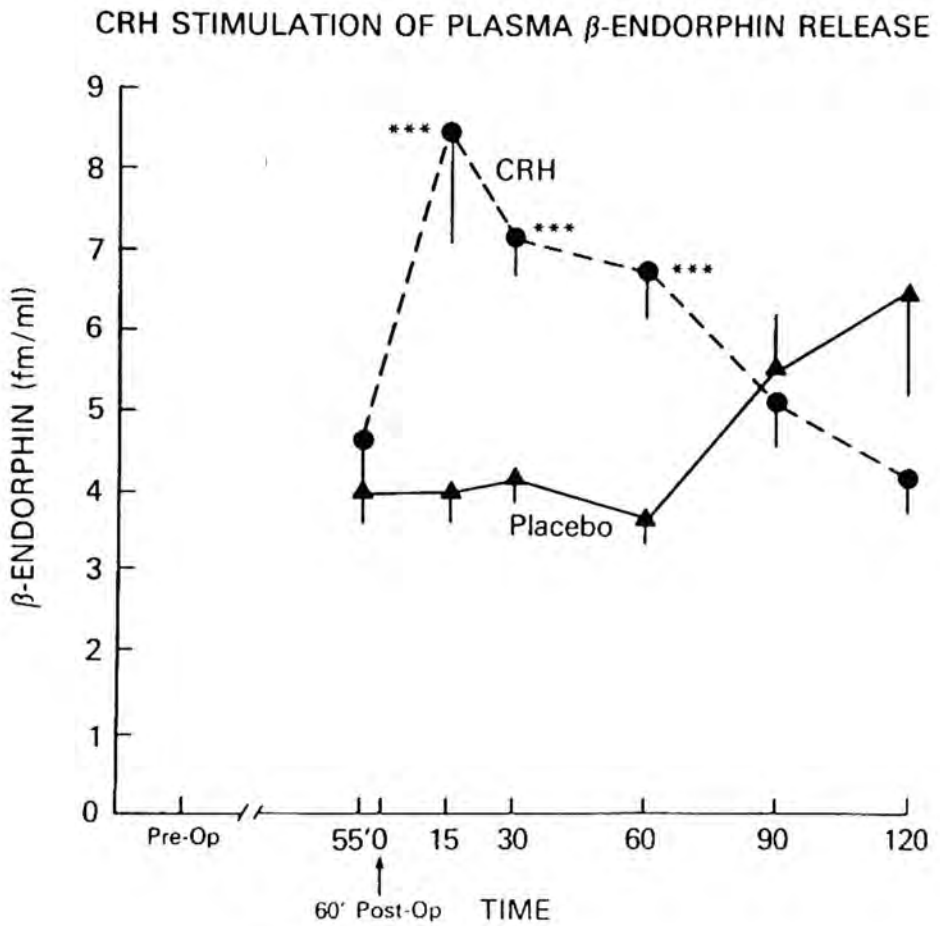
with intravenous premedication. Diazepam (8 of 12 patients) and fentanyl (6 of 12 patients) resulted in frequent occurrences of drowsiness and dizziness, whereas placebo (4 of 12 patients) and naloxone (2 of 12 patients) produced a negligible report of nonspecific effects.

3-1.b. CRH Study

This study determined the effects of post-operative administration of CRH, as compared to a placebo, on the development of acute pain following the surgical extraction of impacted third molars. Figure 3 illustrates the experimental design and sampling schedule for the study. Fourteen patients participated in this cross-over study. The CRH and placebo groups did not significantly differ for surgeon's rating of surgical trauma for mandibular impactions (median scores of 3 vs 2, on a category scale of 1-5, not significant (NS)), for maxillary impactions (1 vs 2, NS), for duration of surgery (12.6 $\pm$ 1.6 min. vs 12.2 $\pm$ 1.7 min., NS), or for offset of local anesthesia. The lack of significant differences for these indices of surgery permits the interpretation of differences in pain as due to drug treatment.

Plasma levels of iB-END varied over time following drug administration (Fig. 10). Circulating levels of iB-END increased, peaking at 15 minutes following administration of CRH. Conversely, administration of

Figure 10. Time course effects of CRH and placebo on circulating levels of iB-END. CRH (1 ug/kg) and placebo were administered 60 minutes after surgery to 14 patients on this cross-over study with samples collected as indicated in Figure 3. *** Significantly different from placebo ($p < 0.001$).


CRH STIMULATION OF PLASMA β-ENDORPHIN RELEASE
β-ENDORPHIN (fm/ml)
9
8
7
6
5
4
3
2
1
0
***
CRH
***
***
Placebo
Pre-Op
55'0
15
30
60
90
120
60' Post-Op
TIME

placebo did not result in a change in plasma iB-END. Plasma levels of iB-END for the CRH group remained elevated as compared to placebo treated patients for up to 60 minutes following administration (Fig. 10). Similar to the endorphin drug study (3-1.a), plasma levels of iB-END increased for the placebo group following the offset of local anesthesia (Fig. 10).

Circulating levels of i-cortisol also changed following drug administration (Fig. 11). The increase in i-cortisol levels peaked 30-60 minutes after CRH administration. The levels of i-cortisol remained greater for the CRH group as compared to the placebo group for 90 minutes following drug administration (Fig. 11).

Contrary to its effect on stimulating the pituitary-adrenal axis, administration of CRH did not alter sympatho-adrenomedullary outflow, as measured by circulating levels of epinephrine and norepinephrine (Fig. 12). Blood-borne epinephrine, depicted in the top panel of Figure 12, did not change following CRH administration. Similarly, norepinephrine levels, illustrated in the bottom panel of Figure 12, were not significantly altered by administration of CRH. However, norepinephrine levels for both groups did significantly increase over time.

The development of post-operative pain was significantly altered by administration of CRH (Fig. 13). Differences in perceived pain did not begin to appear until 90 minutes following drug administration, at a time when

Figure 11. Time course effects of CRH and placebo on plasma levels of i-cortisol. CRH (1 ug/kg) and placebo were administered 60 minutes after surgery to 14 patients using a double-blind cross-over design as indicated in Figure 3. ** Significantly different from placebo ($p < 0.01$).

PLASMA CORTISOL RESPONSES TO CRH ADMINISTRATION

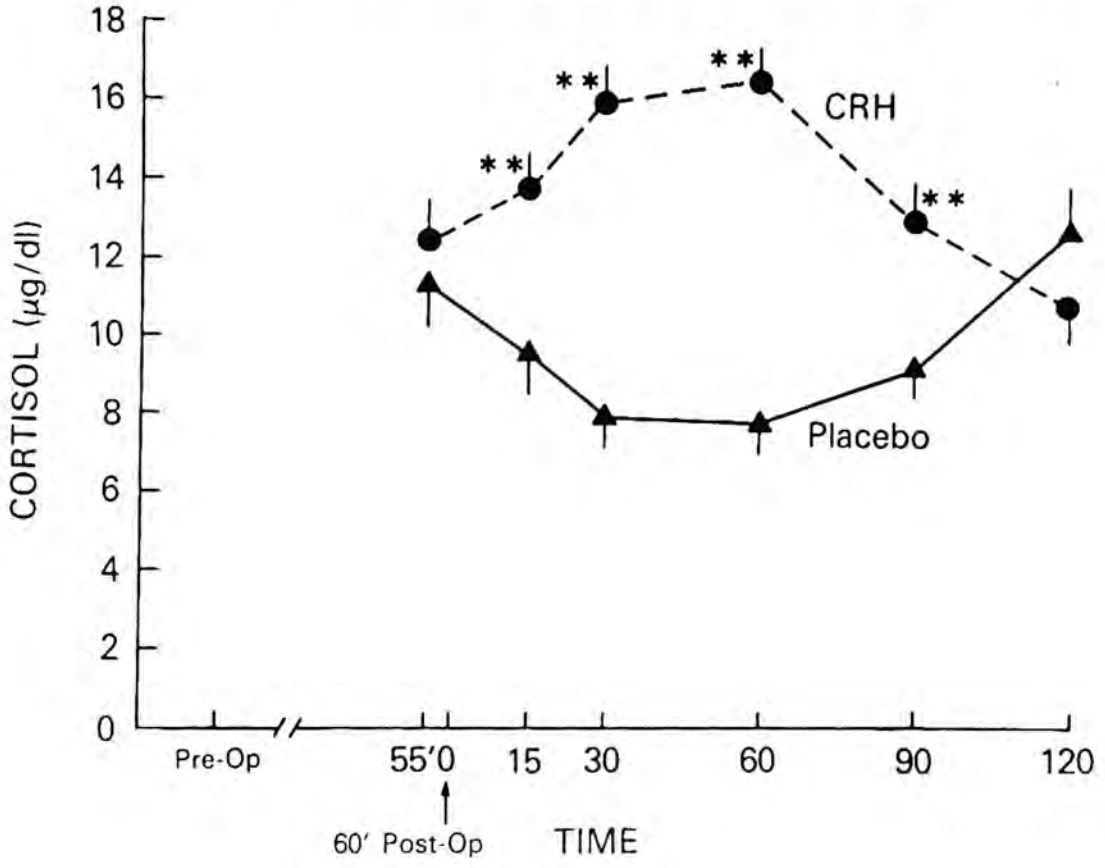


Figure 12. Lack of an effect of CRH or placebo on plasma catecholamines. CRH (1 ug/kg) or placebo were administered to 14 patients 60 minutes following surgery using the experimental design depicted in Figure 3. Top Panel: Circulating levels of epinephrine. ANOVA for repeated measures reveals a lack of significance (time x drug interaction: $F = 1.11$ $p > 0.3$). Bottom Panel: Circulating levels of norepinephrine. ANOVA for repeated measures indicates no significant time x drug interaction ($F = 0.44$, $p > 0.8$). However, norepinephrine levels for both groups did increase over the post-operative sampling period (ANOVA for repeated measures, time: $F = 4.19$, $p < 0.01$).

LACK OF AN EFFECT OF CRH ON PLASMA CATECHOLAMINES

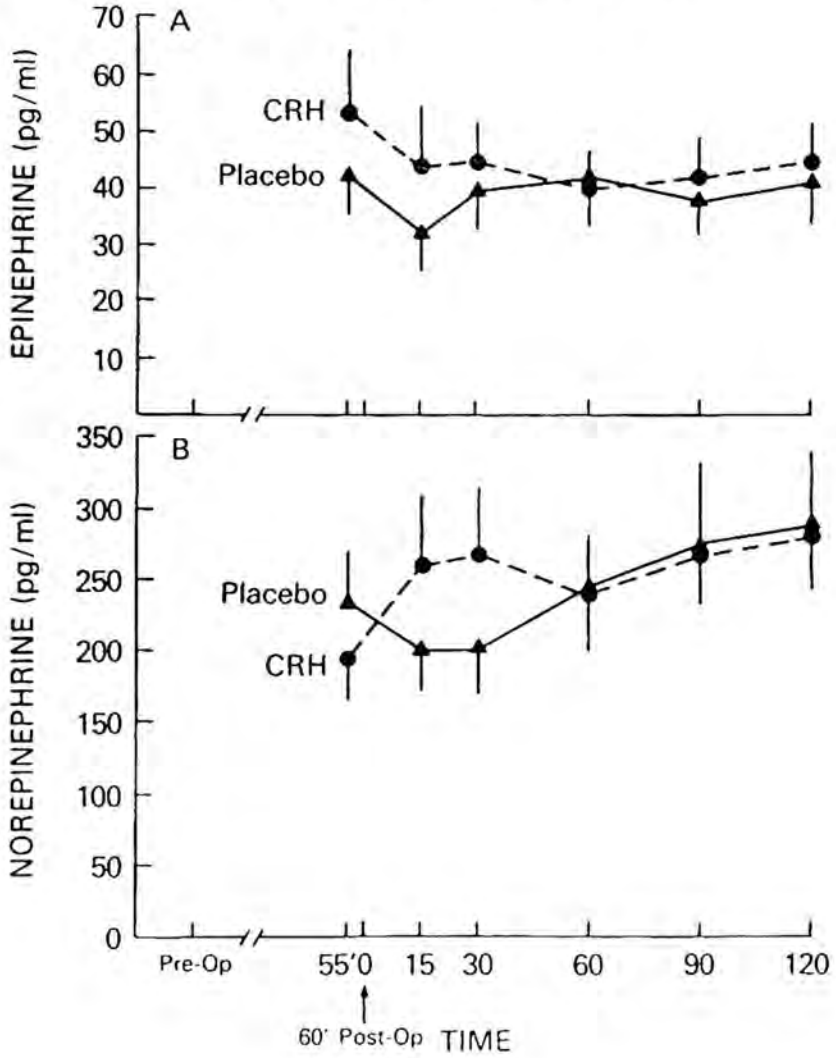
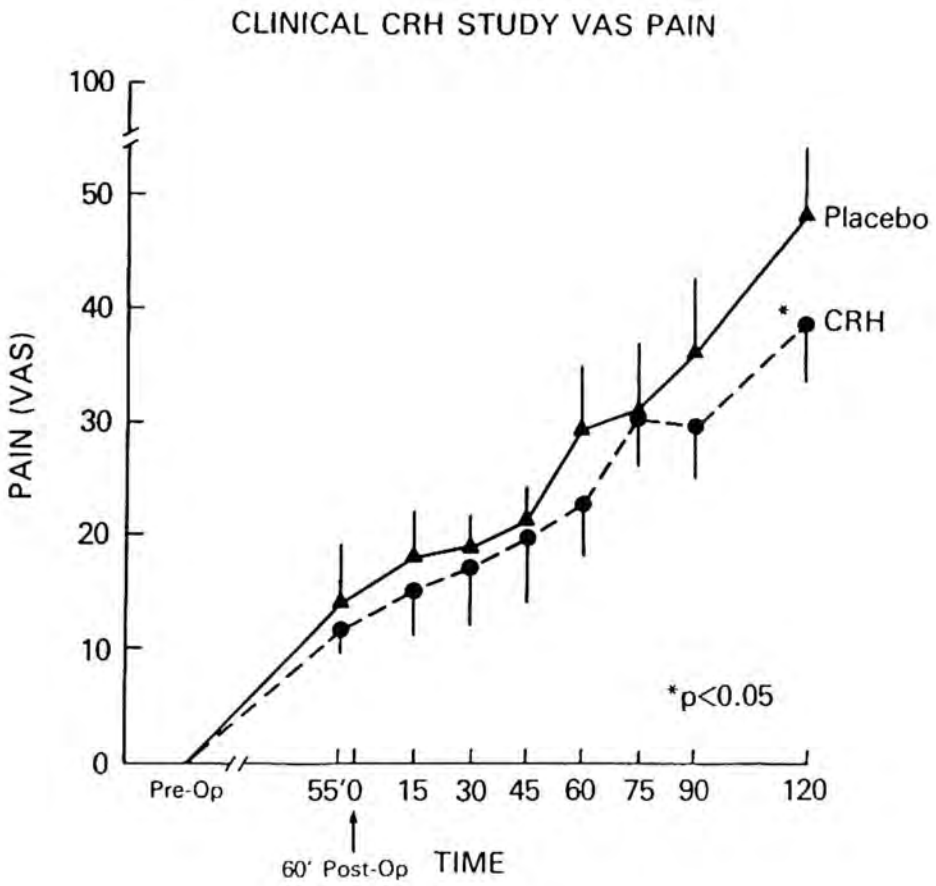


Figure 13. Effects of CRH and placebo on the development of post-operative pain, as measured by a VAS. CRH (1 ug/kg) and placebo were administered to 14 patients at 60 minutes following surgery using a cross-over design as illustrated in Figure 3. * Significantly different from placebo ($p < 0.05$).


CLINICAL CRH STUDY VAS PAIN
PAIN (VAS)
100
50
40
30
20
10
0
Placebo
CRH
*
*p<0.05
Pre-Op
55'0
15
30
45
60
75
90
120
60' Post-Op
TIME

patients reported an offset in local anesthesia. As measured on a VAS scale for pain, CRH treated patients began to report less pain at 90 minutes and reported significantly less pain at 120 minutes following drug administration (Fig. 13).

Two other VAS scales were employed to measure separately the sensory intensity and unpleasantness of pain. Patients administered CRH tended to report a lower sensory intensity of pain at 120 minutes following treatment (Fig. 14), although this did not reach significance ($p = 0.06$). Instead, the primary effect of CRH was a significant reduction in the unpleasantness of pain, with the CRH group reporting significantly less pain at 90 and 120 minutes following treatment (Fig. 15).

Other physiological parameters, selected to measure side effects of CRH treatment, did not alter following administration of CRH (Table 2). CRH had no significant effect on systolic blood pressure, diastolic blood pressure, heart rate, oral temperature or on a VAS for anxiety (Table 2). A facial flushing response was observed in 5 out of 14 patients administered CRH and 3 out of 14 patients administered placebo (NS).

3-1.c. Dexamethasone Study

This study determined the effects of three doses of dexamethasone, as compared to a placebo, on post-operative

Figure 14. Effects of CRH and placebo on the development of the sensory intensity of post-operative pain, as measured by a VAS. CRH (1 ug/kg) and placebo were administered 60 minutes after surgery to 14 patients on this cross-over study with samples collected as indicated in Figure 3.

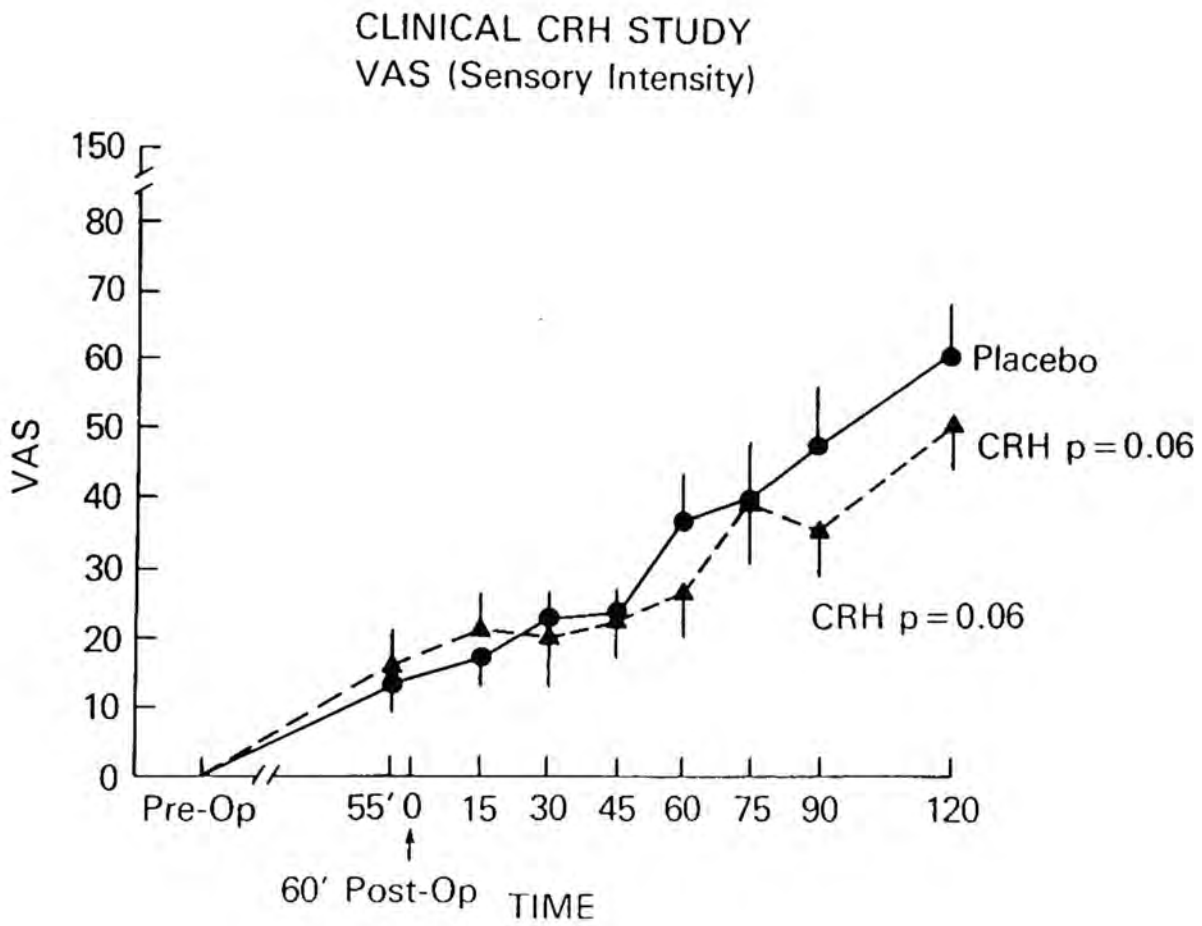
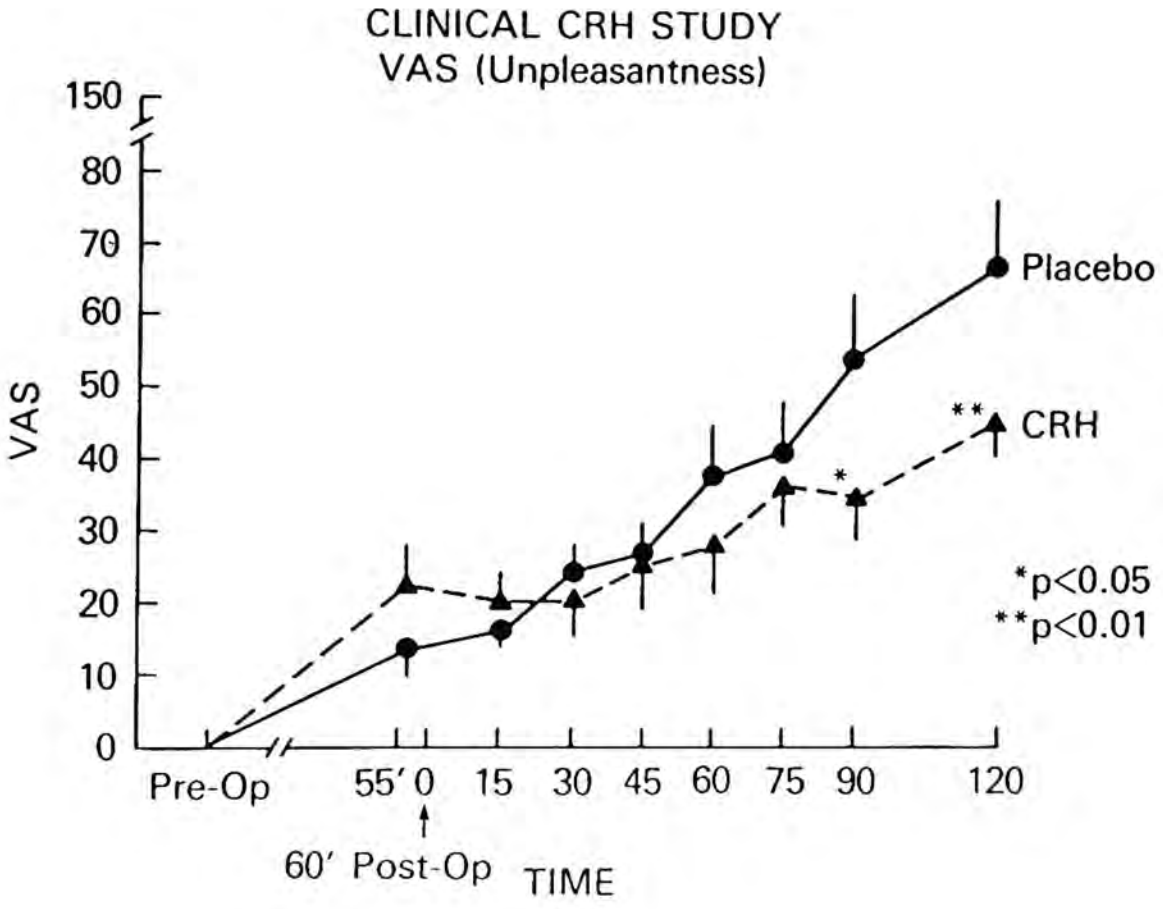

CLINICAL CRH STUDY
VAS (Sensory Intensity)
150
80
70
60
50
40
30
20
10
0
VAS
Placebo
CRH p = 0.06
CRH p = 0.06
Pre-Op
55'
0
15
30
45
60
75
90
120
60' Post-Op
TIME

Figure 15. Effects of CRH and placebo on the development of the unpleasantness of post-operative pain, as measured by a VAS. CRH (1 ug/kg) or placebo were administered 60 minutes following surgery to 14 patients on this cross-over study (Figure 3). * Significantly different from placebo ($p < 0.05$). ** Significantly different from placebo ($p < 0.01$).


CLINICAL CRH STUDY
VAS (Unpleasantness)
VAS
150
80
70
60
50
40
30
20
10
0
Placebo
CRH
**
*
*p<0.05
**p<0.01
Pre-Op
55'
0
15
30
45
60
75
90
120
60' Post-Op
TIME

LACK OF AN EFFECT OF CRH ON ANXIETY AND SELECTED PHYSIOLOGIC PARAMETERS

Parameter	Drug	55'	15'	30'	45'	60'	75'	90'	120'
Anxiety (VAS)	CRH	11.6 ±6.2	5.3 ±2.5	4.5 ±1.6	4.0 ±1.4	9.0 ±5.9	8.3 ±5.6	3.7 ±1.6	3.3 ±1.8
	Placebo	7.8 +3.4	2.5 +1.0	2.8 +1.2	3.4 +1.4	4.5 +1.5	5.5 +2.0	4.6 +1.6	4.8 +1.9
Sys. Bp.	CRH	129 ±3	125 ±4	128 ±3	129 ±3	129 ±2	131 ±2	133 ±2	137 ±3
	Placebo	127 ±2	126 ±3	129 ±3	131 ±3	133 ±4	136 ±4	135 ±4	139 ±6
Dias. Bp.	CRH	77 ±3	73 ±2	74 ±2	75 ±2	76 ±2	78 ±2	81 ±2	82 ±2
	Placebo	74 ±3	75 ±2	75 ±3	78 ±1	80 ±2	80 ±2	78 ±2	76 ±3
Heart Rate	CRH	67 ±2	66 ±2	64 ±2	64 ±2	64 ±2	64 ±2	63 ±2	64 ±2
	Placebo	60 ±3	61 ±2	62 ±3	62 ±3	61 ±3	66 ±2	67 ±3	63 ±3
Oral Temp.	CRH	36.5 ±0.1	36.5 ±0.1	36.4 ±0.1		36.5 ±0.1		36.6 ±0.1	36.6 ±0.1
	Placebo	36.6 ±0.1	36.6 ±0.1	36.7 ±0.1		36.6 ±0.1		36.6 ±0.1	36.6 ±0.1

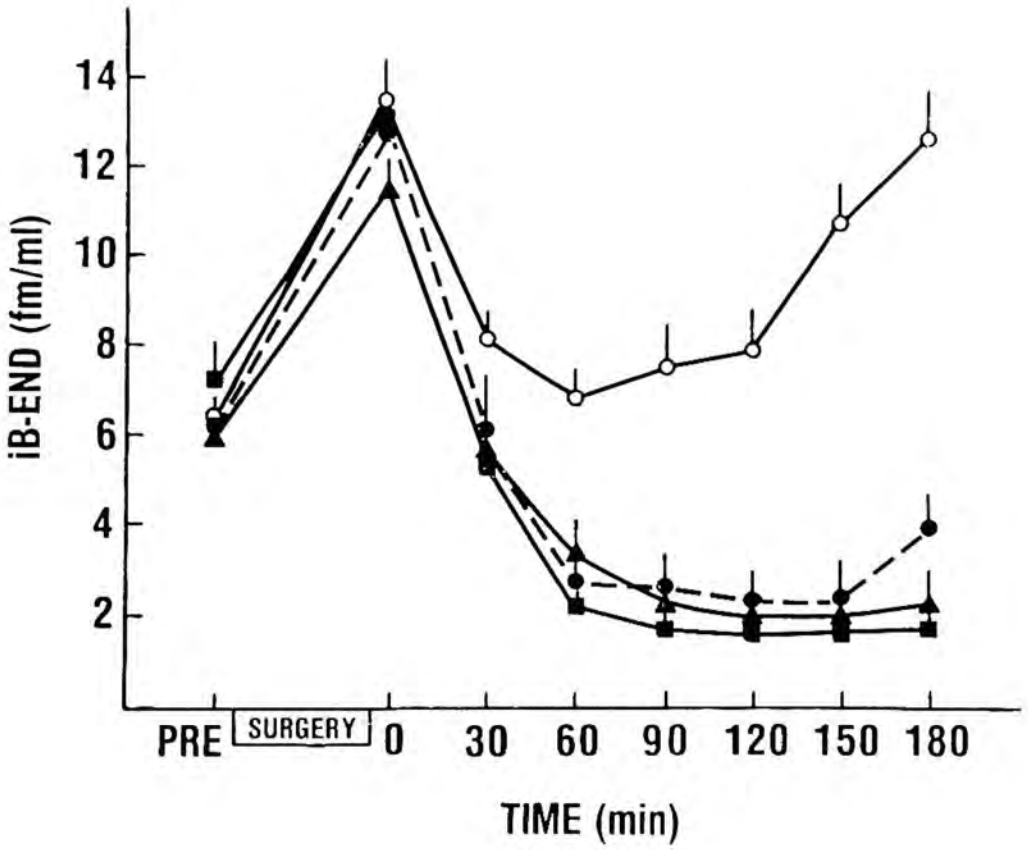
* X + s.e.

pain in forty eight patients following oral surgery. Dexamethasone was administered in doses of 0.1, 0.32 and 1.0 mg at ten minutes following completion of surgery. The experimental design and sampling schedule are presented in Figure 4.

The four groups were quite similar for indices of the surgical experience. The placebo, 0.1 mg, 0.32 mg and 1.0 mg dexamethasone groups were statistically indistinguishable for surgical trauma of maxillary (median scores of 2,2,1,1, respectively) and mandibular (median scores of 2,2,2,2, respectively) third molars. In addition, the duration of surgery for placebo (11.0 $\pm$ 2.0 min.), 0.1 mg dexamethasone (13.0 $\pm$ 2.2 min.), 0.32 mg dexamethasone (13.5 $\pm$ 2.0 min.) and 1.0 mg dexamethasone (10.5 $\pm$ 2.3) did not significantly differ. Finally, all groups reported similar offsets of local anesthesia. The lack of significant differences for these measures of surgery permits the interpretation of differences in pain as due to drug treatment.

Plasma levels of iB-END varied during the experimental period (Fig. 16). In response to the stress of surgery, circulating levels of iB-END nearly doubled for all groups. Plasma levels of iB-END also changed over time following drug administration. From 60 to 180 minutes following drug injection, patients administered dexamethasone, at all three doses, had lower levels of iB-END as compared to placebo treated patients. Similar to

Figure 16. Effects of dexamethasone (0.1, 0.32 and 1.0 mg) and placebo on circulating levels of iB-END. Placebo (open circles), 0.1 mg dexamethasone (closed circles, hatched lines), 0.32 mg dexamethasone (triangles) or 1.0 mg dexamethasone (squares) were administered to patients 10 minutes following completion of oral surgery. From 60 through 180 minutes after drug injection, all groups receiving dexamethasone had significantly lower levels of iB-END as compared to the placebo group ($p < 0.01$).


14
12
10
8
6
4
2
iB-END (fm/ml)
PRE
SURGERY
0
30
60
90
120
150
180
TIME (min)

the previous studies, blood-borne iB-END levels increased for the placebo group following the offset of local anesthesia and the onset of post-operative pain.

Patient reports of pain varied according to drug treatment (Fig. 17). As measured on a VAS scale for pain, patients administered 0.1 mg of dexamethasone reported greater pain than patients given a placebo at 60, 90 and 120 minutes following drug administration (Fig. 17). The group pretreated with 0.32 mg dexamethasone did not differ from the placebo group at any time point, while the 1.0 mg dexamethasone group reported less pain than patients administered placebo at 180 minutes following drug (Fig. 17).

The sensory intensity of pain was also quantitated using a separate VAS scale (Fig. 18). Patients pretreated with 0.1 mg dexamethasone reported greater levels of pain sensory intensity as compared to placebo treated patients at 60, 90 and 120 minutes following drug administration (Fig. 18). The 0.32 mg dexamethasone group appeared statistically indistinguishable from the placebo group, while patients pretreated with 1.0 mg dexamethasone reported less pain intensity than patients administered placebo at 180 minutes following drug administration (Fig. 18).

Similar drug effects were observed on the VAS for the unpleasantness of pain (Fig. 19). The 0.1 mg dexamethasone group demonstrated greater pain

Figure 17. Effects of dexamethasone (0.1, 0.32 and 1.0 mg) and placebo on the development of post-operative pain, as measured by a VAS. The drugs were administered 10 minutes after completion of oral surgery (Figure 4).

* Significantly different from placebo ($p < 0.05$).

** Significnatly different from placebo ($p < 0.01$).

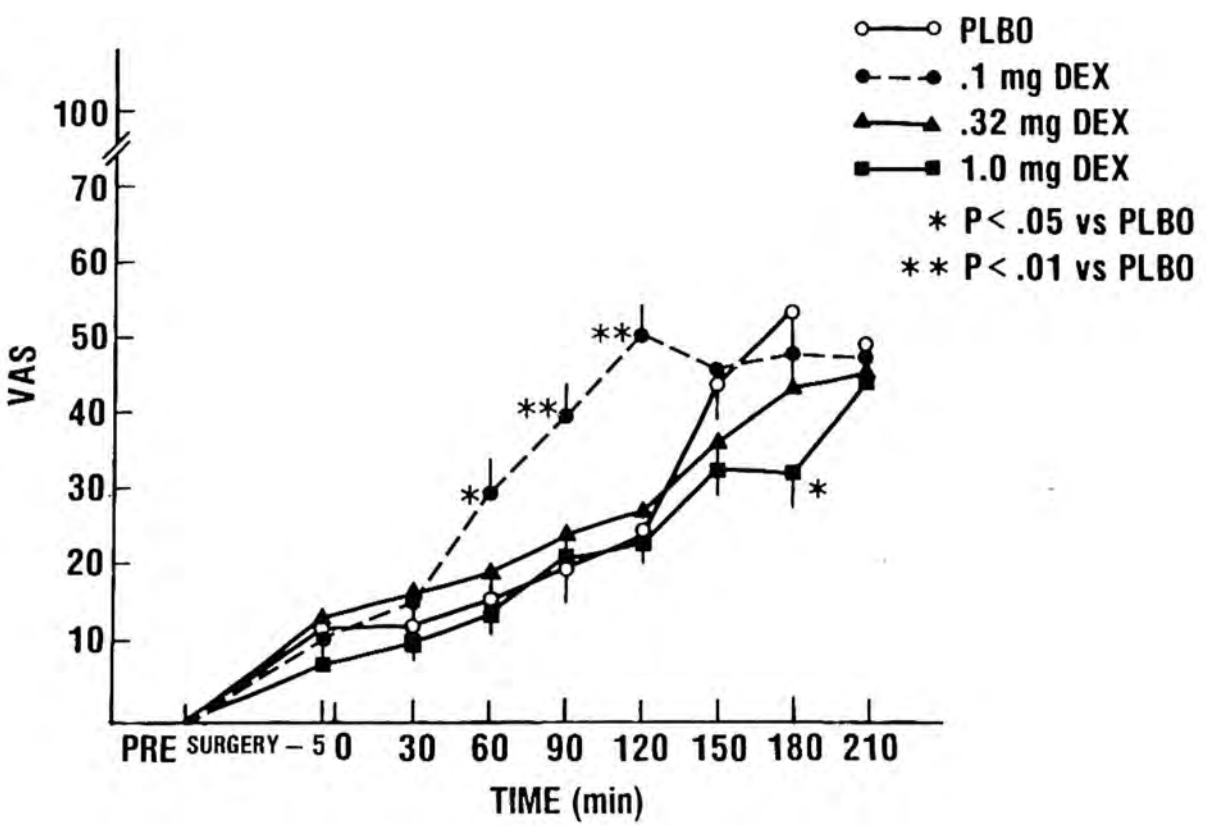

PLBO
.1 mg DEX
.32 mg DEX
1.0 mg DEX
* P< .05 vs PLBO
** P< .01 vs PLBO
VAS
100
70
60
50
40
30
20
10
PRE SURGERY – 5 0 30 60 90 120 150 180 210
TIME (min)

Figure 18. Effects of dexamethasone (0.1, 0.32 and 1.0 mg) and placebo on the development of the sensory intensity of post-operative pain as measured by a VAS. Placebo (open circles), 0.1 mg dexamethasone (closed circles, hatched lines), 0.32 dexamethasone (triangles) or 1.0 mg dexamethasone (squares) were administered to patients 10 minutes following completion of oral surgery.

* Significantly different from placebo ($p < 0.05$).

** Significantly different from placebo ($p < 0.01$).

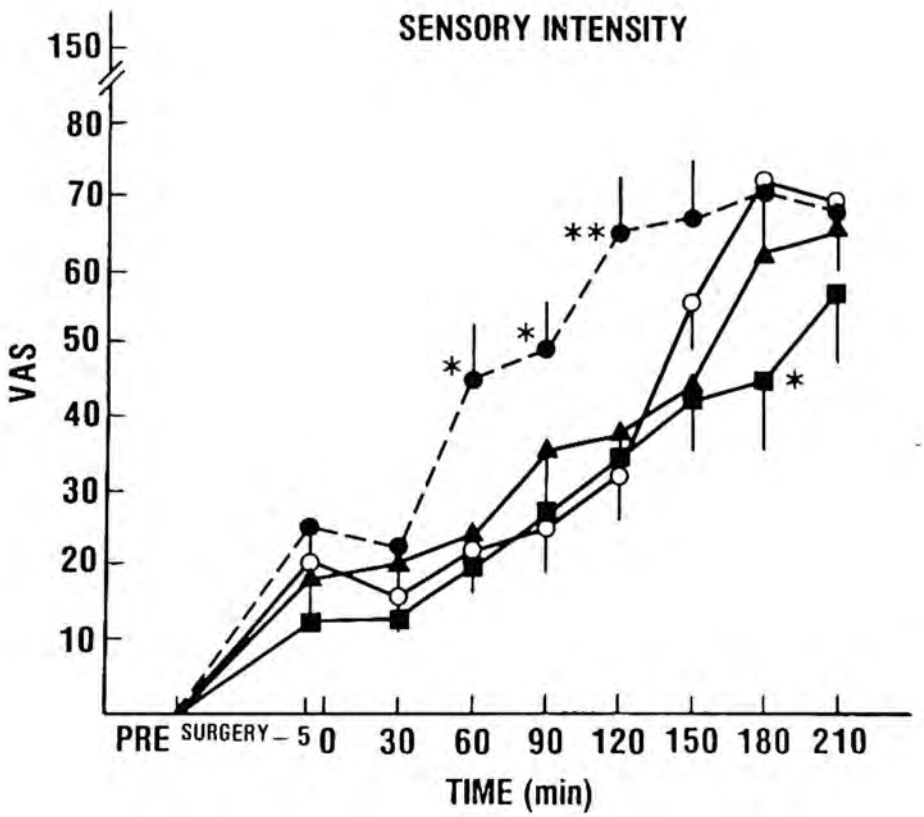
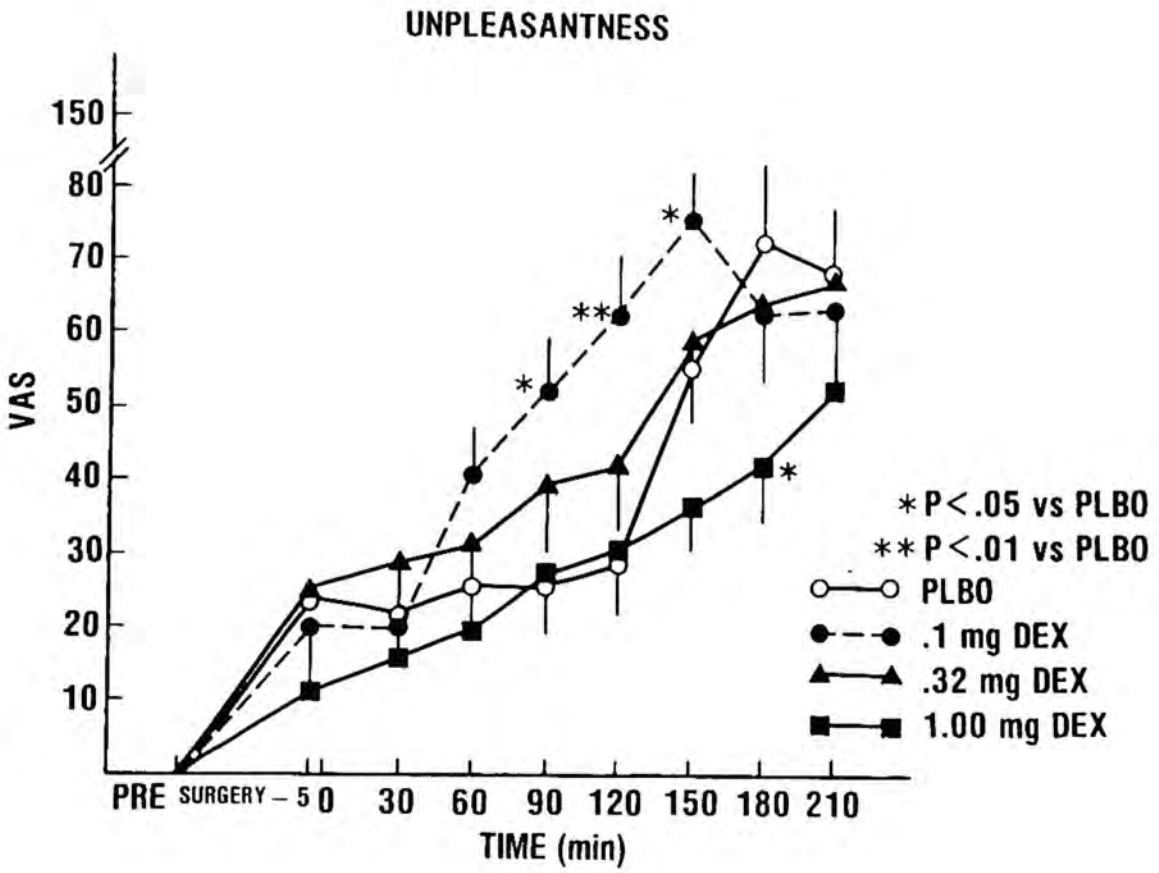

SENSORY INTENSITY
VAS
150
80
70
60
50
40
30
20
10
PRE
SURGERY – 5
0
30
60
90
120
150
180
210
TIME (min)
*
*
**
*

Figure 19. Effects of dexamethasone (0.1, 0.32 and 1.0 mg) and placebo on the development of the unpleasantness of post-operative pain, as measured by a VAS. Drugs were administered 10 minutes following oral surgery (Figure 4).

* Significantly different from placebo ($p < 0.05$).

** Significantly different from placebo ($p < 0.01$).


UNPLEASANTNESS
VAS
150
80
70
60
50
40
30
20
10
PRE
SURGERY – 5
0
30
60
90
120
150
180
210
TIME (min)
* P < .05 vs PLBO
** P < .01 vs PLBO
PLBO
.1 mg DEX
.32 mg DEX
1.00 mg DEX

unpleasantness as compared to the placebo group from 90 minutes through 180 minutes after drug treatment (Fig. 19). The 0.32 mg dexamethasone group did not differ from the placebo group. The 1.0 mg dexamethasone group reported less pain than placebo at 180 minutes following drug administration (Fig. 19).

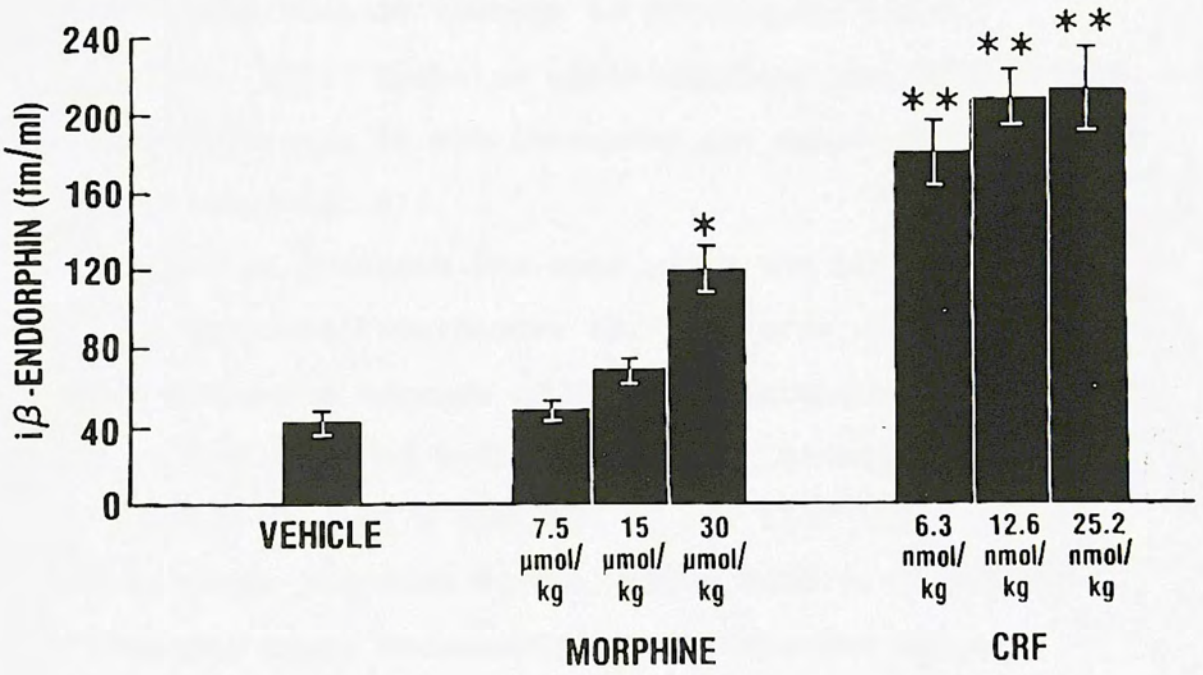
3-2. Animal Experiments

3-2.a. CRH and Morphine Sulfate Dose Response Study

This study determined the optimal dose and time for CRH to produce antinociception. This was compared to the antinociceptive effects of animals concurrently treated with morphine sulfate or vehicle. The experiment employed 8 rats/group. It is important to note that all animal studies were conducted by an observer unaware as to treatment allocation.

Plasma levels of iB-END, collected by a trunk blood procedure 75 minutes following drug administration, varied according to drug treatment (Fig. 20). Administration of CRH, at all three doses, resulted in greater levels of iB-END as compared to the vehicle control (Fig. 20). In contrast, only the high dose of morphine sulfate (30 umol/kg = 10 mg/kg) resulted in greater levels of circulating iB-END as compared to vehicle (Fig. 20).

Figure 20. Effects of CRH, morphine sulfate and vehicle on plasma levels of iB-END in rats. Animals were injected i.v. at time zero, the behavioral antinociception was measured through the next 60 minutes and trunk blood was collected at 75 minutes. N = 8/group. * Significantly different from vehicle ($p < 0.05$). ** Significantly different from vehicle ($p < 0.01$).


iβ-ENDORPHIN (fm/ml)
240
200
160
120
80
40
0
VEHICLE
7.5 µmol/ kg
15 µmol/ kg
30 µmol/ kg
MORPHINE
6.3 nmol/ kg
12.6 nmol/ kg
25.2 nmol/ kg
CRF
*
**
**
**

A change in paw lick latencies over time also occurred in response to drug treatment (Fig. 21). Vehicle treated rats exhibited a decrease in paw lick latency over the 60 minute testing period (Fig. 21). In contrast, rats pretreated with 6.3 nmol/kg CRH had greater latencies as compared to vehicle rats at 30 minutes. Rats treated with 25.2 nmol/kg CRH exhibited a dose related increase in the duration of antinociception, with latencies greater than vehicle observed from 30 through 60 minutes following injection (Fig. 21). Morphine administration resulted in a dose related increase in the intensity and duration of the antinociception (Fig. 21).

Figure 22 presents the area under the time response curve using the data from Figure 21. The area under the curve is a composite measure of both the intensity and the duration of the observed antinociception. Since vehicle treated animals exhibit a decrease in paw lick latency, as compared to their baseline values, their data is presented with a negative sign; conversely drug treatments which produce increases in the latency, as compared to baseline, are presented as positive values. Morphine administration resulted in a dose related increase in the area under the curve at all doses as compared to vehicle (Fig. 22). Administration of CRH produced an apparent biphasic dose-related increase in the area under the curve, with antinociception observed at the 6.2 nmol/kg and 25.2 nmol/kg doses (Fig. 22). These findings prompted selection

Figure 21. Antinociceptive effects of CRH and morphine sulfate following i.v. injections in rats. * Significantly different from vehicle ($p < 0.05$). ** Significantly different from vehicle ($p < 0.01$).

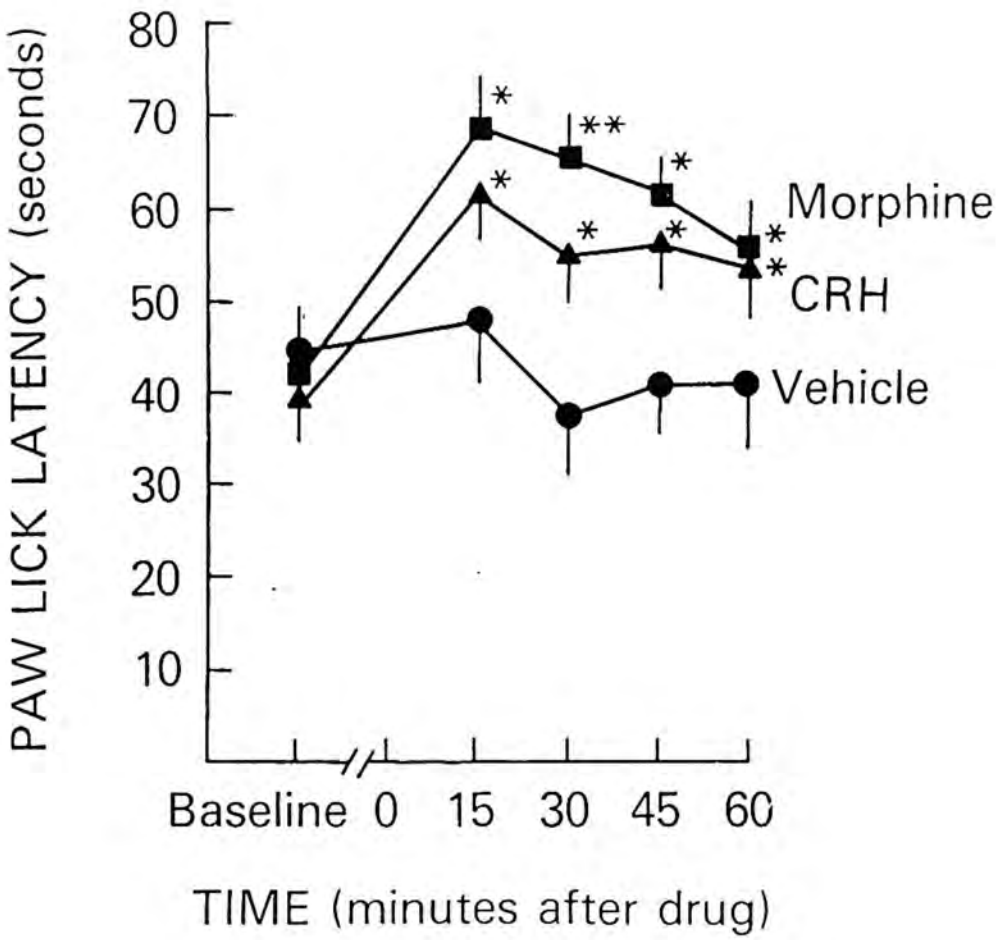
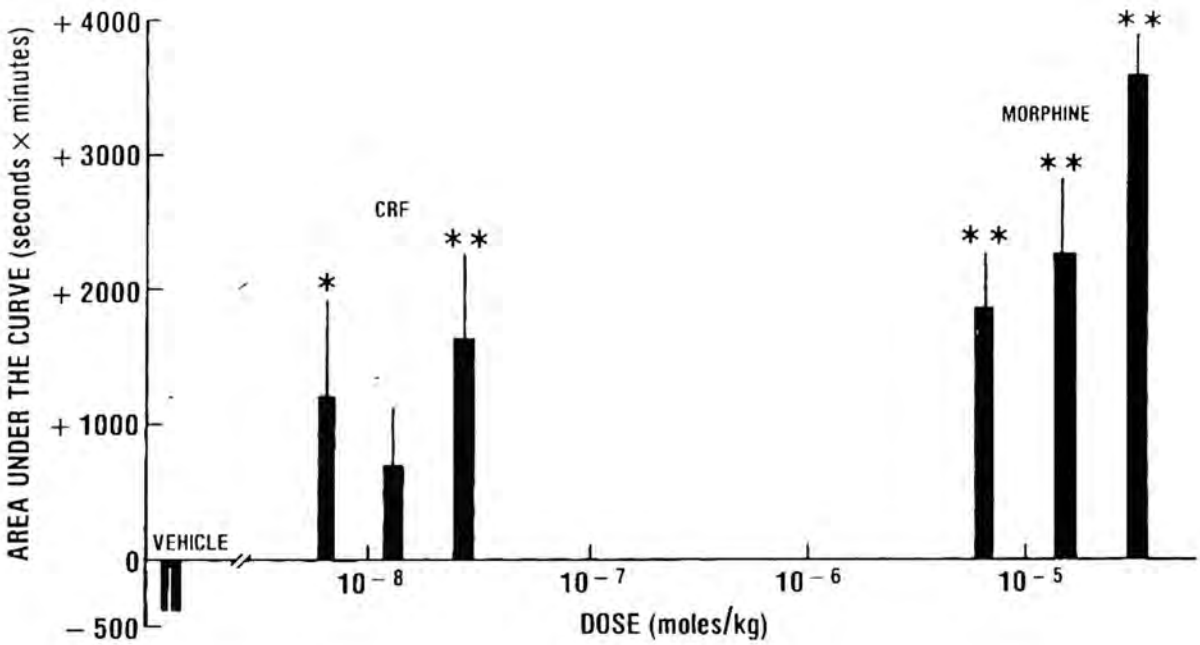

PAW LICK LATENCY (seconds)
80
70
60
50
40
30
20
10
Morphine
CRH
Vehicle
Baseline 0 15 30 45 60
TIME (minutes after drug)

Figure 22. Antinociceptive effects of CRH and morphine sulfate presented as the area under the time response curve. The area under the curve is a composite measure of both the intensity and duration of time response data presented in Figure 21. * Significantly different from vehicle ($p < 0.05$). ** Significantly different from vehicle ($p < 0.01$).


AREA UNDER THE CURVE (seconds × minutes)
+ 4000
+ 3000
+ 2000
+ 1000
0
− 500
VEHICLE
CRF
MORPHINE
*
**
**
**
**
10 − 8
10 − 7
10 − 6
10 − 5
DOSE (moles/kg)

of the 25.2 nmol/kg dose of CRH for subsequent studies directed at determining the participation of pituitary B-END in mediating CRH-induced antinociception.

3-2.b. Naltrexone Study

This study determined the effects of opiate receptor blockade on CRH-induced antinociception by using a double injection paradigm. Eight rats/group were administered i.v. one of the following five treatment combinations: vehicle/vehicle, vehicle/CRH, 1 mg/kg naltrexone/CRH, 10 mg/kg naltrexone/CRH or 10 mg/kg quaternary naltrexone/CRH. The quaternary naltrexone employed was naltrexone methylbromide.

Pretreatment with naltrexone did not alter pituitary corticotroph responsiveness to subsequent administration of CRH (Fig. 23). As compared to the vehicle/vehicle group, rats treated with vehicle/CRH had a 5 fold increase in plasma levels of iB-END at 75 minutes following drug administration (159 $\pm$ 23 fm/ml vs 30 $\pm$ 2 fm/ml). Similar plasma iB-END levels were observed in CRH rats pretreated with either 1 mg/kg naltrexone, 10 mg/kg naltrexone or 10 mg/kg quaternary naltrexone (Fig. 23); all had greater plasma iB-END levels than rats administered vehicle/vehicle.

Administration of naltrexone did block CRH-induced antinociception (Fig. 24). Rats administered the

Figure 23. Lack of an effect of naltrexone (NAL) and a quaternary naltrexone (Q-NAL) on CRH-induced stimulation of plasma levels of iB-END in rats. Trunk blood was collected 75 minutes following i.v. injection of drugs. N = 8/group. ** Significantly different from vehicle (VEH) ($p < 0.01$).

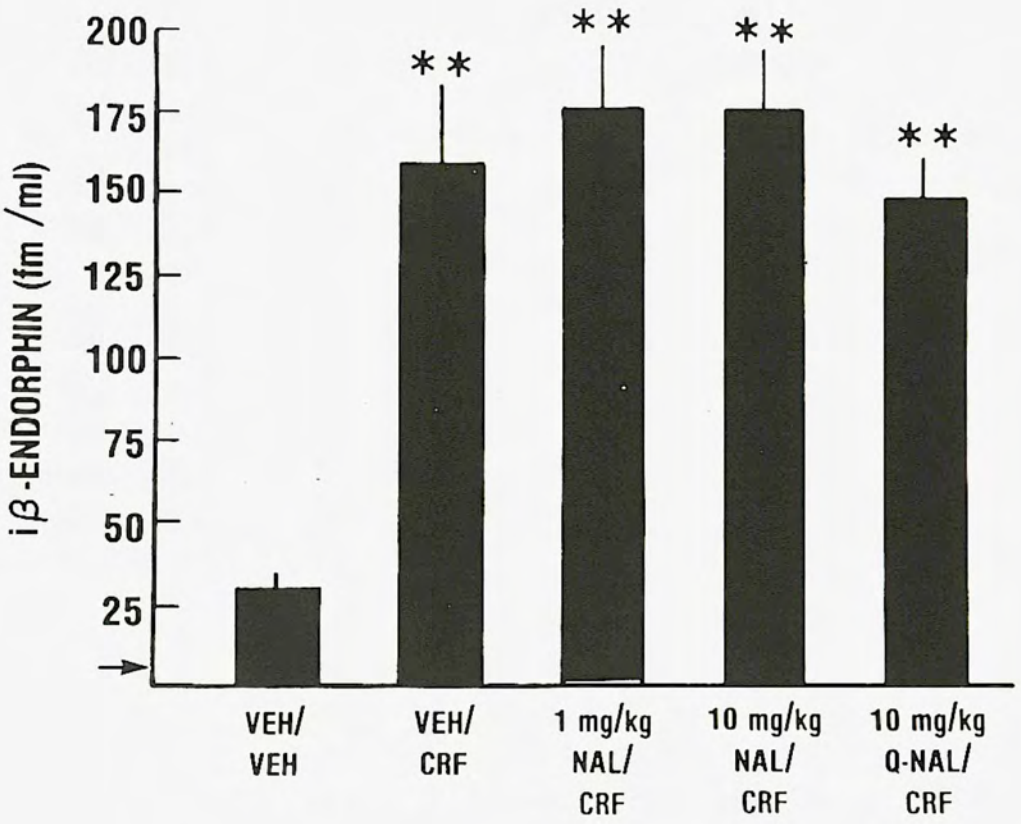
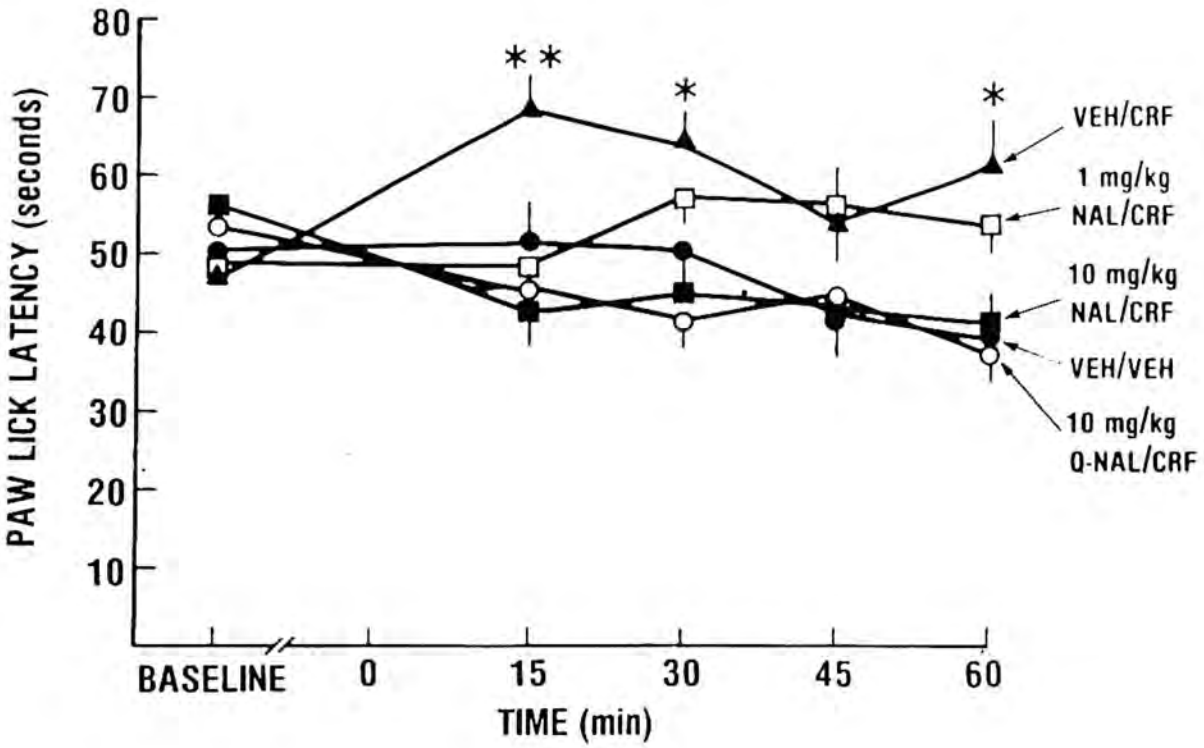

200
175
150
125
100
75
50
25
iβ-ENDORPHIN (fm /ml)
**
**
**
**
VEH/ VEH
VEH/ CRF
1 mg/kg NAL/ CRF
10 mg/kg NAL/ CRF
10 mg/kg Q-NAL/ CRF

Figure 24. Blockade of CRH-induced antinociception by pre-treatment with naltrexone (NAL) and a quaternary naltrexone (Q-NAL). * Significantly different from vehicle/vehicle (VEH/VEH) treated animals ($p < 0.05$). ** Significantly different from vehicle/vehicle treated animals ($p < 0.01$).


80
70
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40
30
20
10
PAW LICK LATENCY (seconds)
**
*
*
VEH/CRF
1 mg/kg
NAL/CRF
10 mg/kg
NAL/CRF
VEH/VEH
10 mg/kg
Q-NAL/CRF
BASELINE
0
15
30
45
60
TIME (min)

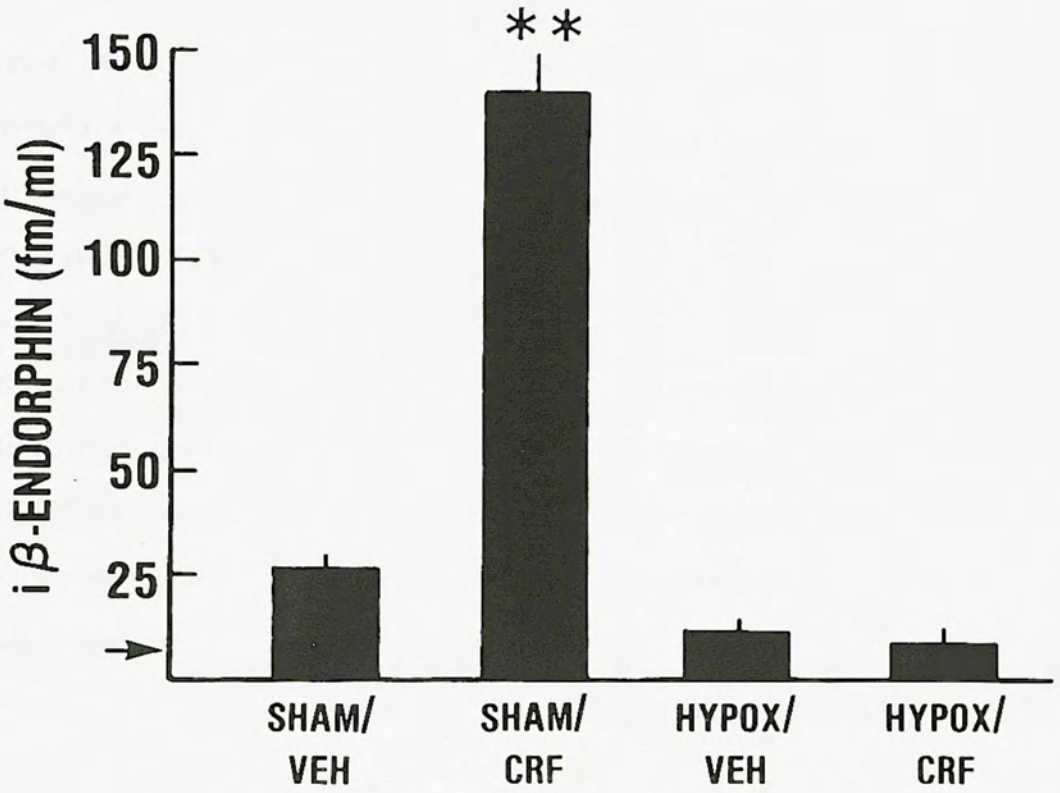
combination vehicle/CRH exhibited antinociception, as compared to the vehicle/vehicle group, at 15, 30 and 60 minutes following drug injection (Fig. 24). In contrast, all three naltrexone/CRH groups were indistinguishable from vehicle/vehicle at 15 minutes and were significantly less than the vehicle/CRH group. From 30 minutes to 60 minutes following drug treatment, the 1 mg/kg naltrexone/CRH group exhibited a time related increase in paw lick latency approaching that of the vehicle/CRH group (Fig. 24). However, as seen in Figure 24, both the 10 mg/kg naltrexone/CRH and 10 mg/kg quaternary naltrexone/CRH groups continued to have latencies similar to vehicle and together these were less than vehicle/CRH at 15, 30 and 60 minutes.

3-2.c. Hypophysectomy Study

This study determined the necessity of an intact pituitary gland for the development of CRH-induced antinociception by employing hypophysectomized (hypox) rats and control rats who underwent a sham surgery (sham). Ten rats/group were employed in this 2 X 2 factorial design and assigned to one of the following four groups: sham/vehicle, sham/CRH, hypox/vehicle, hypox/CRH.

The effects of these treatment combinations on plasma iB-END levels are presented in Figure 25. Administration of CRH to sham rats resulted in

Figure 25. Effect of hypophysectomy (HYPOX), as compared to a sham surgery (SHAM) on CRH-induced stimulation of plasma levels of iB-END in rats. Trunk blood was collected 75 minutes following i.v. injection of CRH or vehicle (VEH). Arrow indicates the minimal detection limit of the RIA. ** Significantly different from SHAM/VEH ($p < 0.01$).


i β-ENDORPHIN (fm/ml)
150
125
100
75
50
25
**
SHAM/ VEH
SHAM/ CRF
HYPOX/ VEH
HYPOX/ CRF

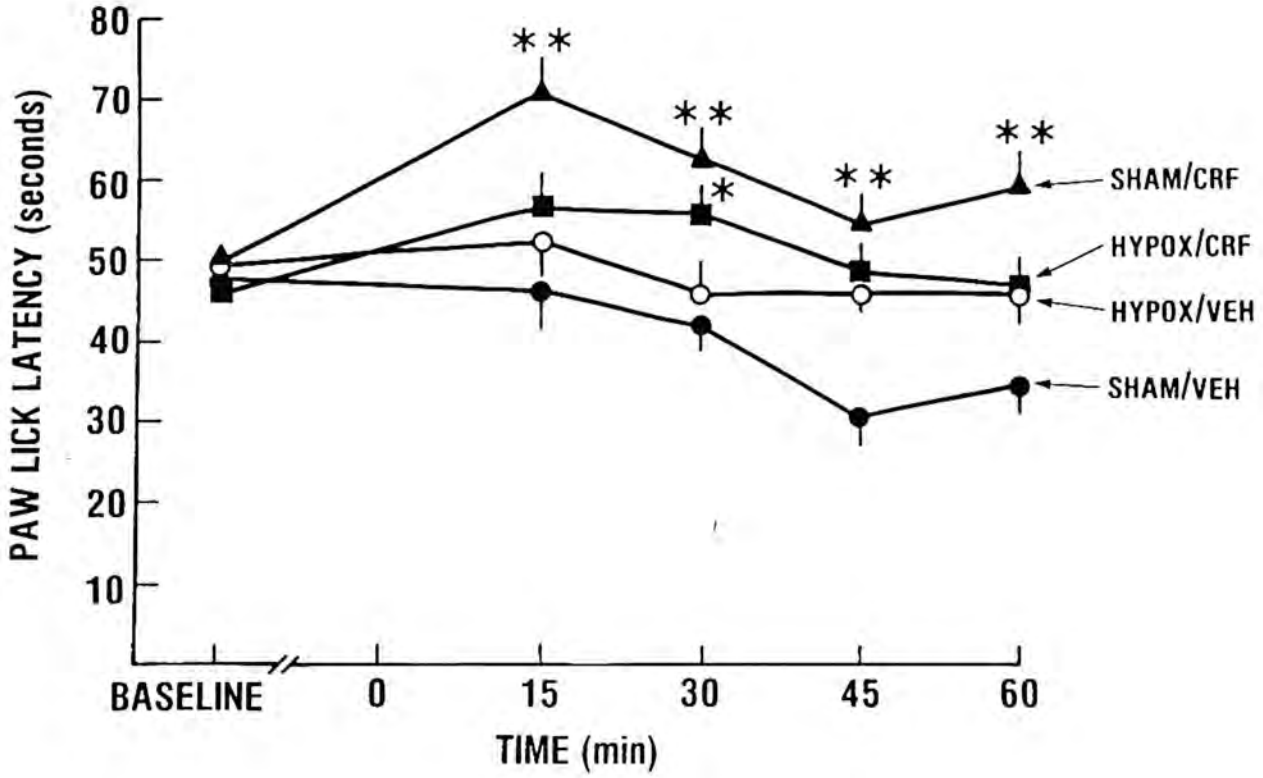
approximately a 5-fold increase in plasma levels of iB-END as compared to vehicle treated sham rats. In contrast, hypophysectomy completely blocked the increase in plasma iB-END which would normally occur following injection of CRH (Fig. 25). Plasma levels of iB-END in both the hypox/vehicle and hypox/CRH groups were near the minimal detection limit of the RIA.

The behavioral nociception of the animals was also affected by the treatment combinations (Fig. 26). Sham rats pretreated with CRH exhibited greater paw lick latencies, as compared to sham/vehicle rats, at 15, 30, 45 and 60 minutes following injection (Fig. 26). In contrast, CRH had little effect in hypophysectomized rats, although it did differ from hypox rats given vehicle at 30 minutes. The sham/CRH group had greater paw lick latencies than the hypox/CRH group at 15 and at 60 minutes. Interestingly, hypox/vehicle rats exhibited a fairly stable paw lick latency over the 60 minute test period as compared to the decrease in latencies observed in sham/vehicle rats (Fig. 26).

3-2.d. Dexamethasone Study

The dexamethasone study tested the requirement of functional pituitary corticotrophs for the development of CRH-induced antinociception. Ten rats/group were assigned to one of the following four treatment combinations:

Figure 26. Blockade of CRH-induced antinociception by hypophysectomy (HYPOX), as compared to sham surgery (SHAM). * Significantly different from respective control ($p < 0.05$). ** Significantly different from respective control ($p < 0.01$).


80
70
60
50
40
30
20
10
PAW LICK LATENCY (seconds)
**
**
**
**
*
SHAM/CRF
HYPOX/CRF
HYPOX/VEH
SHAM/VEH
BASELINE
0
15
30
45
60
TIME (min)

vehicle/vehicle, vehicle/CRH, dexamethasone/vehicle and dexamethasone/CRH.

Plasma levels of iB-END varied according to the treatment conditons (Fig. 27). Circulating levels of iB-END in the vehicle/CRH treated rats were approximately 8-fold higher than in the vehicle/vehicle control group. Pretreatment with dexamethasone blocked the stimulatory effect of CRH on pituitary corticotrophs and in fact, suppressed control levels by approximately 36% (i.e., dexamethasone/vehicle vs vehicle/vehicle).

Paw lick latencies were also modified by the treatment combinations (Fig. 28). Rats pretreated with vehicle and then administered CRH exhibited greater latencies than the vehicle/vehicle group at 15, 30 and 45 minutes following drug administration (Fig. 28). In contrast, the dexamethasone/CRH group demonstrated latencies which appeared statistically indistinguishable from the dexamethasone/vehicle control group (Fig. 28). In addition, the latencies of the vehicle/CRH group were significantly greater than dexamethasone/CRH at both 15 and 45 minutes following injection.

3-2.e. Passive Immunization Study

The next study determined the effect of passively immunizing rats with anti-endorphin antisera on the development of CRH-induced antinociception. Ten rats/group

Figure 27. Effect of pre-treatment with dexamethasone (DEX), as compared to vehicle (VEH), on CRH-induced stimulation of plasma levels of iB-END in rats. Dexamethasone (0.5 mg/kg), or vehicle were administered i.p. two hours before i.v. injection of CRH or vehicle. Trunk blood was collected 75 minutes following injection of CRH or vehicle. Arrow indicates the minimal detection limit of the RIA. ** Significantly different from vehicle/vehicle treated rats ($p < 0.01$).

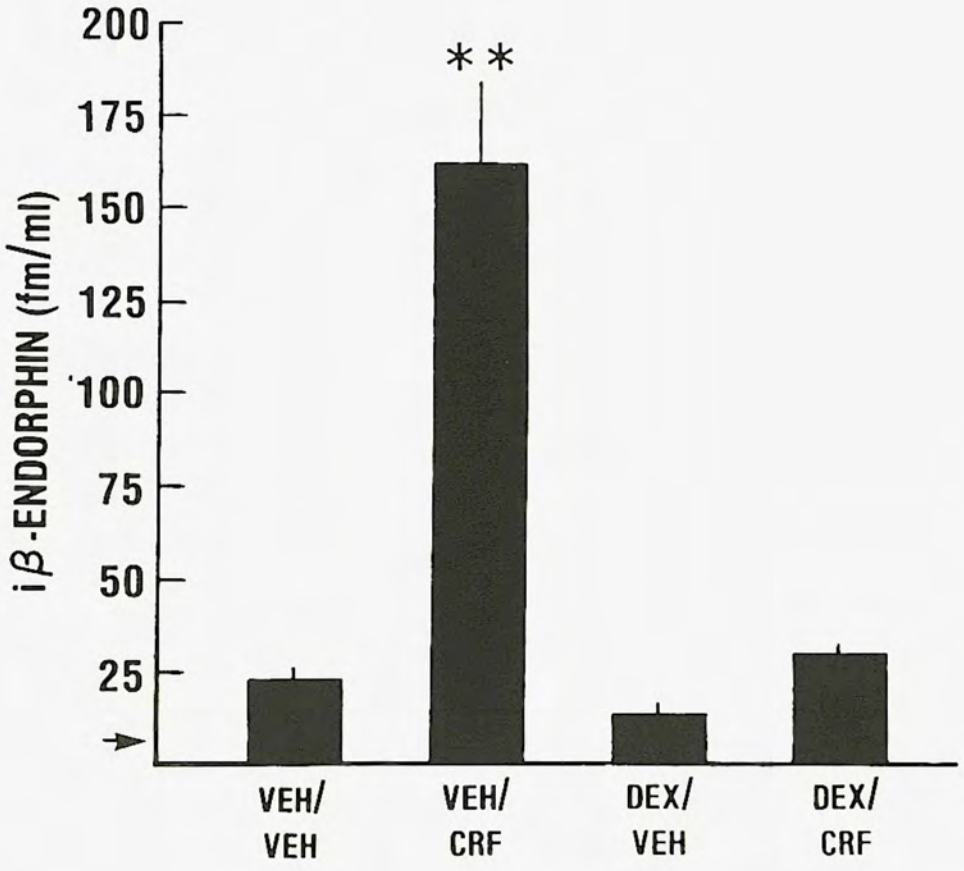
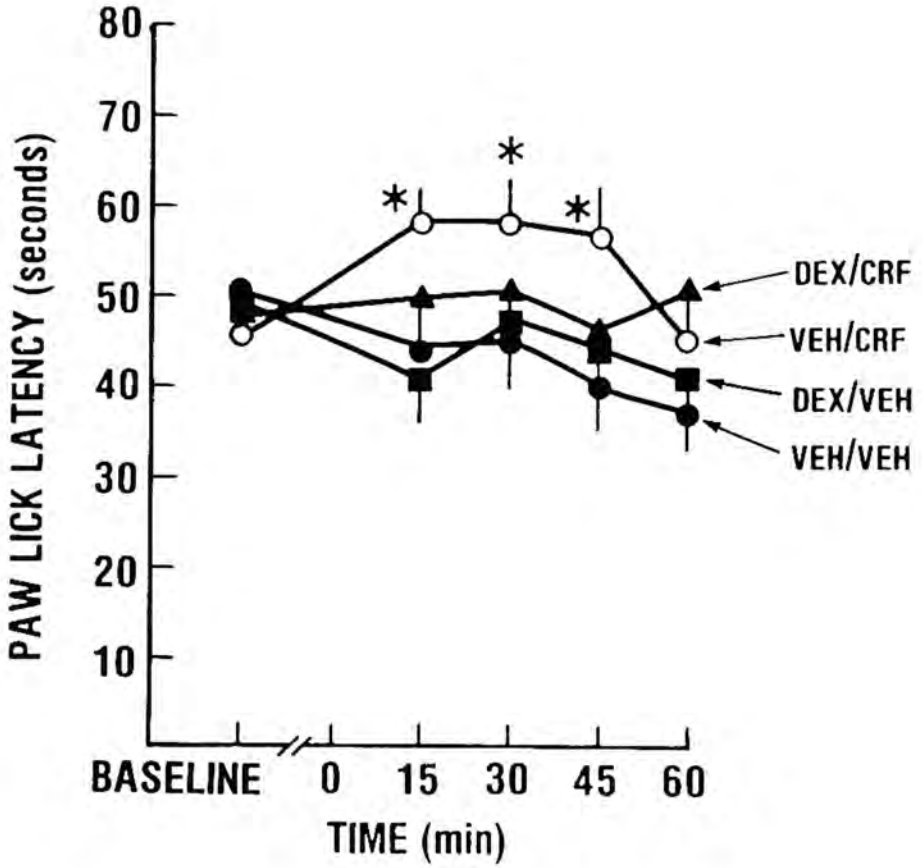

200
175
150
125
100
75
50
25
i β-ENDORPHIN (fm/ml)
**
VEH/
VEH
VEH/
CRF
DEX/
VEH
DEX/
CRF

Figure 28. Blockade of CRH-induced antinociception by pre-treatment with dexamethasone (DEX) as compared to vehicle (VEH). * Significantly different from respective control ($p < 0.05$).


80
70
60
50
40
30
20
10
PAW LICK LATENCY (seconds)
*
*
*
DEX/CRF
VEH/CRF
DEX/VEH
VEH/VEH
BASELINE
0
15
30
45
60
TIME (min)

were assigned to one of the following four treatment combinations: hyperimmune antisera/vehicle, hyperimmune antisera/CRH, anti-endorphin antisera/vehicle or anti-endorphin antisera/CRH. However, several preliminary studies were conducted to maximize the information obtained and to exclude potential confounds in interpretation which may occur due to passive immunization.

The first preliminary study determined the lack of cross-reactivity between anti-endorphin antisera and CRH. CRH did not displace tracer B-END from the anti-endorphin antisera, even at CRH concentrations three-fold in excess of blood levels calculated to occur immediately after i.v. injection. The lack of detectable cross-reactivity implies that anti-endorphin antisera blockade of CRH-induced antinociception is not due to the sequestering of CRH.

The second preliminary study determined the effect of peripherally administered passive immunization on the antinociceptive activity of centrally (i.c.v.) administered B-END. In this study, 7 rats/group were first given a peripheral injection of either saline, hyperimmune antisera or anti-endorphin antisera, and then given a central (i.c.v.) injection of either $B\text{-}END_{1\text{-}31}$ (10 ug in 10 ul over 1 min.) or saline (10 ul). At 30 minutes following injection, the latencies for the treatment combinations of saline/B-END (88.2 $\pm$ 7.1 seconds), hyperimmune/B-END 77.3 $\pm$ 11.8 seconds) and anti-endorphin/B-END (86.8 $\pm$ 8.4 seconds) did not differ. In addition, the latencies for all three

groups were significantly greater than the saline/saline group (39.2 $\pm$ 6.5 seconds, $p < 0.01$ for all three groups).

The third preliminary study determined that the binding capacity (fm iB-END bound) was linearly related to the amount of the anti-endorphin antisera. Three RIA standard curves were incubated with differing concentrations of antisera. From each of these curves, the amount of unlabeled B-END required to cause a 50% reduction in binding was obtained. This amount of B-END, when multiplied by the % binding of the zero tube, gives the actual amount of B-END bound to the antisera. For example, suppose that 100 ul of diluted antisera binds to 30% of the trace (i.e., zero tube) and has a 50% reduction in binding with 10 fm of B-END. The actual amount of B-END bound to the antisera is (0.30 X 10 fm = 3.0 fm). For the standard curves, the amount of antisera in each curve was plotted against the amount of B-END bound. This plot of binding capacity vs amount of antisera was subjected to linear regression analysis and gave a correlation coefficient of 0.999 ($p < 0.01$). Thus, the amount of B-END bound is linearly related to the amount of antisera available. This relationship was exploited in the passive immunization study.

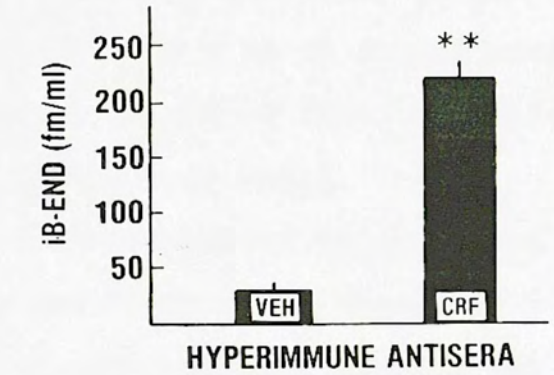
In this study, rats were passively immunized with anti-endorphin antisera and, following collection of the behavioral data, trunk blood was obtained. The amount of free antisera still remaining in the blood was determined

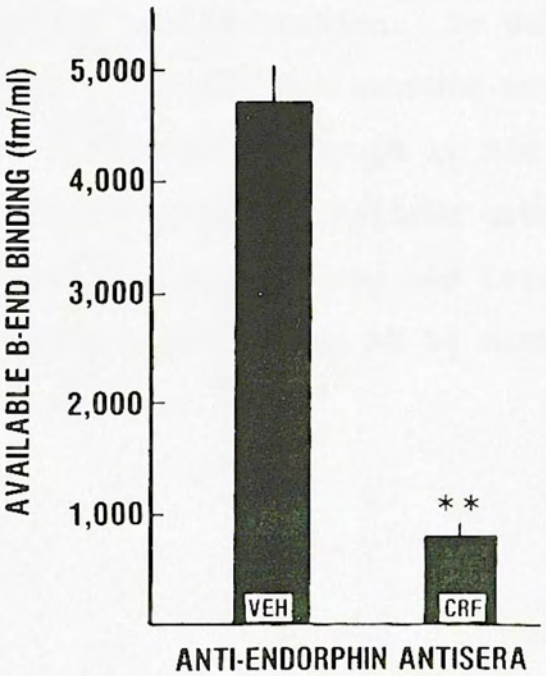
by conducting a binding assay of 5 dilutions of the plasma sample (1:10 to 1:1,000). The binding assay simply determined the % binding of the plasma aliquot to the B-END trace. Samples with more available (i.e., free) antisera had a greater % binding to the trace. The actual amount of free antisera in the sample was determined by comparing the % binding of the plasma sample to a "standard curve" consisting of 9 concentrations of antisera. Taken together, the % binding of a plasma sample from a passively immunized rat was used to determine the amount of free antisera circulating in the animal and this information was then converted into the available iB-END binding (fm iB-END binding/ml plasma).

In the actual experiment, administration of CRH to animals passively immunized with hyperimmune serum resulted in significantly greater levels of circulating iB-END as compared to rats treated with the combination of hyperimmune antisera/vehicle (Fig. 29 top panel). In passively immunized anti-endorphin rats, increases in circulating iB-END leads to decreases in available antisera, since most of the antisera has been bound by blood-borne iB-END. As seen in Figure 29 (bottom panel), administration of CRH to passively immunized anti-endorphin rats resulted in a significant decrease in the amount of available B-END binding as compared to anti-endorphin/vehicle treated rats. The difference in available antisera binding between the two treatments can

Figure 29. Effects of passively immunizing rats with hyperimmune antisera (top panel) or anti-endorphin antisera (bottom panel) on CRH-induced stimulation of plasma levels of iB-END in rats. Top Panel: Plasma levels of iB-END for rats pre-treated with hyperimmune antisera and then administered either CRH or vehicle (VEH). Bottom Panel: Plasma levels of free anti-endorphin antisera, in units of femtomoles of available binding sites of iB-END/ml plasma, in rats pre-treated with anti-endorphin antisera and then administered either CRH or vehicle. The arrow indicates the level of blood-borne iB-END in the hyperimmune/CRH group.

** Significantly different from respective control ($p < 0.01$).

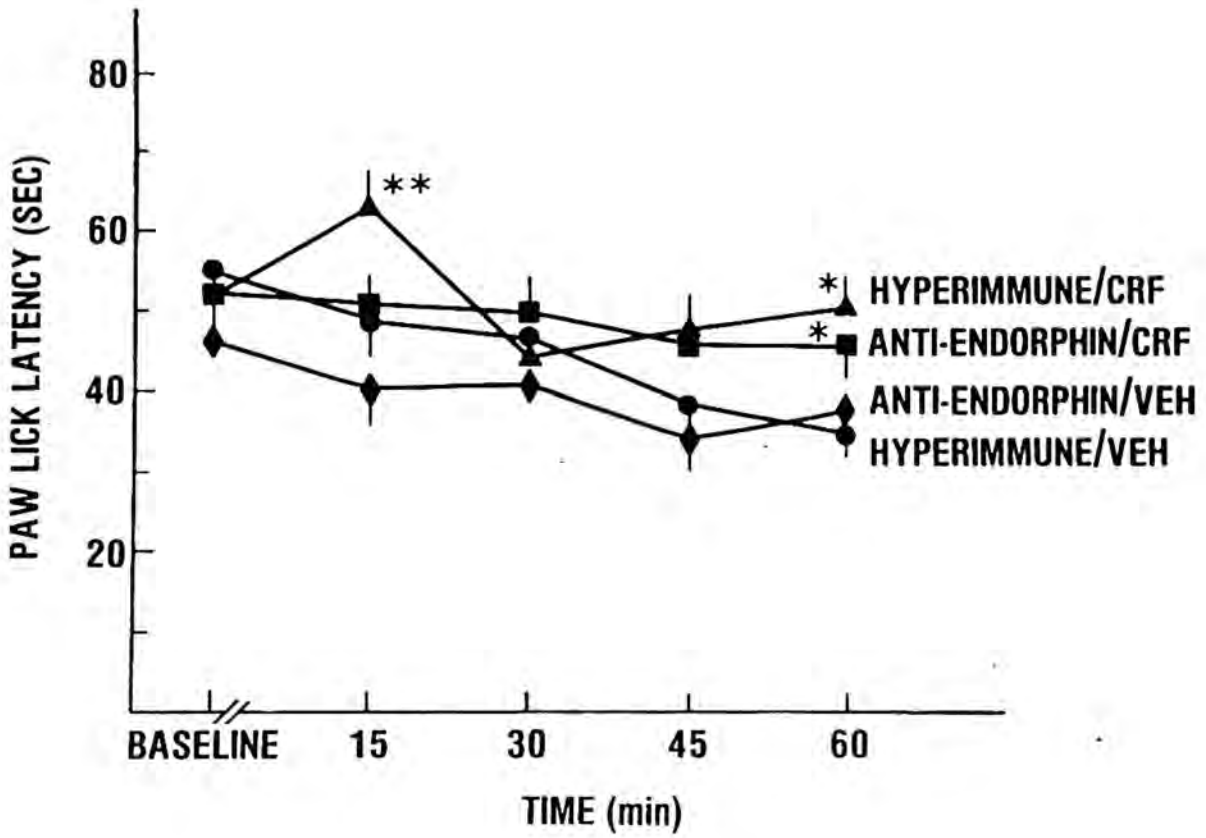

iB-END (fm/ml)
250
200
150
100
50
**
VEH
CRF
HYPERIMMUNE ANTISERA


AVAILABLE B-END BINDING (fm/ml)
5,000
4,000
3,000
2,000
1,000
**
VEH
CRF
ANTI-ENDORPHIN ANTISERA

be interpreted to indicate the total amount of iB-END secreted by the pituitary corticotrophs. Thus, the pituitary corticotroph secretes about 3900 fm iB-END/ml plasma/75 minutes in response to CRH. In addition, the presence of free antisera remaining in the anti-endorphin/CRH group (848 ± 95 fm iB-END binding/ml plasma) implies that actual amount of free, or biologically active, iB-END is most likely very small.

The effects of passive immunization on CRH-induced antinociception are depicted in Figure 30. The hyperimmune/CRH group exhibited greater paw lick latencies, as compared to the hyperimmune/vehicle group at both 15 and 60 minutes following administration. In contrast, the anti-endorphin/CRH group did not exhibit any increases over its own baseline latencies, although it did have a latency greater than the anti-endorphin/vehicle group at 60 minutes. The hyperimmune/CRH group had latencies greater than the anti-endorphin/CRH group at 15 minutes following drug administration (Fig. 30).

Figure 30. Effect of passively immunizing rats with either anti-endorphin antisera or hyperimmune antisera on CRH-induced antinociception. * Significantly different from respective control ($p < 0.05$). ** Significantly different from respective control ($p < 0.01$).


80
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PAW LICK LATENCY (SEC)
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*
*
HYPERIMMUNE/CRF
ANTI-ENDORPHIN/CRF
ANTI-ENDORPHIN/VEH
HYPERIMMUNE/VEH
BASELINE
15
30
45
60
TIME (min)

CHAPTER 4

DISCUSSION

4-1. Overview

This project tested the hypothesis of an analgesic action of pituitary B-END through administration of CRH to both humans and rats. The physiologic relevance of the research lies in the fact that the potential role of pituitary B-END in modifying nociception was evaluated directly by the specific and selective stimulation of pituitary corticotroph secretion by CRH.

Summarized briefly, the principle findings from the clinical studies were: 1) that oral surgery and acute post-operative pain constitute physiologically relevant stressors which resulted in significant alterations in pituitary secretion of iB-END and which activated an endogenous opioid pain suppression system, as indicated by a naloxone-induced hyperalgesia; 2) that stimulation of pituitary corticotrophs by CRH suppressed post-operative pain; and 3) that inhibition of pituitary corticotrophs by a low dose of dexamethasone increased post-operative pain.

The animal studies determined that the release of pituitary B-END mediates CRH-induced antinociception. Specifically, these studies revealed that: 1) opiate antagonists; 2) hypophysectomy; 3) dexamethasone suppression of pituitary corticotroph activity; and 4) passive immunization with anti-endorphin antisera all decreased the ability of CRH to produce analgesia.

Together, these findings demonstrate that CRH is analgesic in both humans and rats and that this is due to the release of B-END from the pituitary gland.

4-2. Clinical Studies

4-2.a. Selection of Oral Surgery as a Clinical Model to Determine Analgesic Actions of Pituitary B-END: Validation of the Model by the Endorphin Drug Study

The results from the endorphin drug study indicate that oral surgery constitutes a physiologically relevant model of stress, with significant increases in circulating B-END occurring both during surgical stress and acute post-operative pain. Equally important, the demonstration of naloxone hyperalgesia indicates that these same conditions activate endogenous opioid analgesic systems.

It is generally appreciated that the oral surgery model is a sensitive clinical bioassay for evaluating the analgesic and anxiolytic efficacy of drugs (Troullos, et

al. 1986, Cooper 1983). The present findings extend the utility of this model and indicate that it is an appropriate paradigm to determine if pituitary B-END modulates the perception of pain. The observation that naloxone, fentanyl and diazepam altered both subjective perceptions of stress and pituitary B-END secretion which occurred in response to surgery, implies that pituitary B-END is physiologically relevant. Importantly, naloxone hyperalgesia was associated with a compensatory increase in circulating levels of iB-END.

An additional finding was the significant increase in intra-operative pain reported by naloxone-pretreated patients as compared to patients administered placebo. To our knowledge, this is the first study to determine the effect of naloxone during surgery, when pain is minimal due to local anesthesia. The use of independent visual analog and verbal descriptor scales for pain strengthen this finding. In addition, the verbal descriptor scales, which measure separately the sensory intensity and unpleasantness of pain, revealed that the naloxone effect was due to a difference in the perceived unpleasantness of the noxious stimuli, rather than a difference in the magnitude of the sensory intensity. The finding that naloxone more than doubled the perception of pain, as compared to placebo, suggests that endogenous opioid peptides may play a significant role in minimizing the perception of intra-

operative pain even in the presence of concurrent local anesthesia.

Several previous studies have demonstrated naloxone-induced hyperalgesia during post-operative pain (Lasagna, et al. 1965, Levine, et al. 1978, 1979, Gracely, et al. 1983). These studies do not exclude the possibility that naloxone hyperalgesia may be due to mechanisms unrelated to opioid antagonism (Hayes, et al. 1977, Sawynor, et al. 1979). However, the present study did not reveal any effect of naloxone on anxiety or on patient report of non-specific side effects. Thus, naloxone's hyperalgesic effects appear attributable to alterations in pain perception rather than a generalized analeptic effect.

Both surgical stress and stress from acute post-operative pain appear sufficient to evoke release of endogenous opioid peptides which are capable of altering the perception of pain. These data are consistent with findings that naloxone is without effect in pain-free individuals (Grevert and Goldstein 1978), but can alter experimental pain when preceded by a stressor (Willer, et al. 1981). These results are interpreted to indicate that endogenous opioid systems are not tonically active. Rather, opioid mechanisms involved in the perception of pain, as reflected by the presence of a naloxone-induced hyperalgesia, are activated under conditions of stress. The results from this and previous studies employing the oral surgery model (Levine, et al. 1978, 1979, Gracely, et

al. 1983) indicate that these periods of opioid activation occur during surgical stress and acute post-operative pain.

The stress of surgery in patients administered placebo also produced a significant increase in plasma levels of norepinephrine. The present results indicate that the factors which regulate plasma levels of iB-END and plasma levels of norepinephrine differ, since naloxone enhanced and fentanyl inhibited the iB-END response to surgical stress without having parallel effects on plasma norepinephrine. These findings demonstrate that secretion of pituitary B-END and activation of the sympathetic nervous system are regulated separately despite their common response to stress.

The results of the study indicate that increases in circulating B-END occur at times when naloxone is capable of eliciting pain. However, further studies were required to determine the existence of a functional relationship between B-END and the perception of pain. These studies are discussed in the following sections.

4-2.b. Effects of Stimulating the Pituitary-Adrenal Axis on Post-Operative Pain

The results from the clinical CRH study indicate that stimulation of pituitary corticotrophs results in a significant inhibition in the development of post-operative pain.

In response to administration of CRH, circulating levels of iB-END approximately doubled within 15 minutes. In addition, the levels of iB-END persisted above placebo levels for 60 minutes following CRH. Administration of CRH stimulated the entire axis, as evidenced by increases in circulating i-cortisol which peaked 60 minutes following drug administration. The delay in peak cortisol levels, in contrast to the rapid increases observed for B-END, is due to the fact that glucocorticoids must be synthesized prior to their secretion (Baxter and Tyrrell 1981). Thus, CRH caused a rapid and sustained activation of the pituitary-adrenal axis.

Following the offset of local anesthesia, patients pretreated with CRH reported significantly less pain than placebo treated patients. These differences cannot be ascribed to group differences in surgical trauma, duration of surgery or offset of local anesthesia. In addition, it is not certain that the maximal analgesic effect of CRH was observed, since the two groups continued to separate at the end of the observation time. The difference in pain was due primarily to significantly less unpleasantness reported in the CRH group.

Differential effects of drugs on the sensory intensity and unpleasantness of pain have been noted for some time. For example, opiates are reported to have an analgesic effect primarily by decreasing the patients perception of the unpleasantness of pain (Jaffee and Martin

1985). Thus, the analgesic effects of CRH were similar to opiates like morphine with respect to decreasing the unpleasantness of pain.

The results of the present study demonstrate the specificity of the biological actions of CRH. In contrast to its effects on stimulating pituitary corticotrophs, CRH did not alter sympatho-adrenomedullary outflow, as measured by circulating catecholamines (see above). This finding, similar to the data from the drug study, provides an additional example of the dissociation between stimuli that activate the pituitary adrenal axis as compared to those that stimulate sympatho-adrenomedullary outflow. In addition, CRH did not alter patient reports of anxiety. Thus, the CRH effect appears quite specific for activation of pituitary corticotrophs and relief of post-operative pain.

The delay in the development of CRH analgesia probably relates to the mechanism by which CRH acts. The time course of the pain response is consistent with most pituitary endocrine phenomena, which are generally slow in onset and prolonged in duration. Further, if penetration of circulating B-END across the BBB was an essential step in the development of analgesia, than substantially prolonged times might be expected (Rapoport, et al. 1980). This is a common issue in pharmacology, since penetration across biological membranes is often a major determinant for the onset of the observed biological effect. For

example, the peak in morphine analgesia is observed 1-2 hours following parenteral administration; at the time when blood levels have fallen to 16-36% of their peak levels (Grabinski and Kaiko 1986).

The delay in the onset of CRH analgesia may also have been due to a lack of bioassay sensitivity at the early time points. It is evident that detection of an analgesic effect requires the presence of pain. However, due to ethical and practical considerations, the drugs were administered at a time when patients were reporting pain at 13% of maximal. The groups did not begin to separate until pain was about 33% of maximal. Thus, an early drug effect may have been obscured due to an initial lack of bioassay sensitivity.

Standing alone, this clinical study does not provide evidence for the mechanism of CRH induced analgesia. In the present surgical model which evaluates inflammatory pain, CRH analgesia may be due to any of several possible mechanisms. First, CRH analgesia could be due to pituitary secretion of B-END, a hypothesis supported by the animal studies, discussed below. Second, CRH stimulation of the entire pituitary adrenal axis resulted in significant increases in circulating glucocorticoids, which may exert anti-inflammatory effects and thus produce analgesia (Flower, et al. 1986 see discussion for dexamethasone study below). Third, CRH could act centrally to stimulate the release of opioid peptides within the

brain. And fourth, CRH may exert a direct effect of its own which is independent of endogenous opioids. This effect might occur either in the spinal cord or in peripheral nerves (Skofitsch, et al. 1985, Kiang and Wei 1985). The most probable of these hypotheses, release of endogenous opioids, could be tested in a clinical trial by administration of CRH followed by naloxone.

From 60 to 120 minutes after drug administration, plasma levels of iB-END increased in placebo treated patients, while levels decreased in patients administered CRH. This may be due either to a subjective difference between the two groups, or to an endocrine difference or to a combination of the two. The two groups markedly differed in their subjective state, since patients administered placebo reported significantly more pain than patients administered CRH. As the endocrine response is probably related to the magnitude of the stress, patients reporting more pain (i.e., placebo group) would be expected to have higher levels of circulating iB-END. In addition, the CRH group had experienced significantly greater levels of i-cortisol, which could suppress subsequent pituitary corticotroph responsiveness to endogenous CRH.

The primary findings of the clinical CRH study are that stimulation of the pituitary-adrenal axis is associated with significantly lower levels of post-operative pain, primarily due to a decrease in the perceived unpleasantness of pain. The reduction in pain in

patients treated with CRH was preceded by increased circulating levels of iB-END.

4-2.c. Effects of Inhibiting the Pituitary-Adrenal Axis on Post-Operative Pain

The role of B-END in analgesia was further evaluated by inhibiting its release with dexamethasone, a synthetic glucocorticoid. The clinical study evaluated the effects of three doses of dexamethasone, as compared to a placebo, on circulating levels of iB-END and patient reports of post-operative pain. Thus, a dose related inhibition of B-END levels and exacerbation of post-operative pain was anticipated.

Administration of dexamethasone 10 minutes following completion of surgery blocked increases in plasma iB-END levels as compared to placebo treated patients. Suppression of the pituitary-adrenal axis was observed at all doses. As in the previous clinical studies, the placebo group had maximal increases in circulating iB-END following the offset of local anesthesia and the onset of acute post-operative pain.

Contrary to what was expected, dexamethasone did not have a linear effect on post-operative pain. While the low dose was consistent with the hypothesis that blockade of pituitary B-END release could result in increased clinical pain, the high dose of dexamethasone was actually

found to be analgesic, not hyperalgesic. It is important to note that these differences in pain cannot be explained by group differences in surgical trauma, duration of surgery or offset of local anesthesia. Patients treated with the low dose of dexamethasone (0.1 mg) reported significantly greater pain, which at 120 minutes was about double that reported by placebo treated patients. This effect of the low dose of dexamethasone is similar to the hyperalgesia noted following administration of naloxone in the drug study (see above). The group receiving the intermediate dose of dexamethasone (0.32 mg) did not significantly differ from placebo over the observation period, whereas patients administered the high dose (1.0 mg) reported significantly less pain than patients administered placebo. Thus, low doses of dexamethasone are hyperalgesic, moderate doses do not separate from placebo and higher doses are analgesic.

The most reasonable explanation for the findings of the higher dose seems related to the anti-inflammatory properties of dexamethasone, which inhibit pain at its source. Glucocorticoids exert a profound dose-related suppression of most known biochemical and immunological mediators of inflammation (Baxter and Tyrrell 1981, Munck, et al. 1984, Flower, et al. 1986) and these actions most likely underlie the analgesic effects of the 1.0 mg dose of dexamethasone observed in this study. By contrast, the low dose of dexamethasone appears to have produced hyperalgesia

through a suppression of pituitary B-END release which was not accompanied by a significant reduction in inflammation.

This conclusion is based upon the assumption that pituitary corticotroph secretion is more sensitive to the inhibitory action of dexamethasone as compared to oral inflammation. This appears to be a reasonable assumption since doses of dexamethasone used to assess the function of the pituitary adrenal axis (i.e., dexamethasone suppression test) are considerably lower than those used to treat inflammation (Baxter and Tyrell 1981, Haynes and Larner 1985). Thus, low doses of dexamethasone have a major effect on suppressing pituitary corticotrophs with little clinically evident anti-inflammatory action. Accordingly, low doses of dexamethasone are prone to result in hyperalgesia under conditions of inflammatory pain. Conversely, higher doses of dexamethasone which exert both pituitary corticotroph suppression and anti-inflammatory actions, can have opposite effects on post-operative pain, with occurrence of either hyperalgesia or analgesia depending on which effect predominates.

Taken together, data from the CRH and dexamethasone studies indicate that stimulation of pituitary corticotrophs is associated with a decrease in patient report of post-operative pain, while suppression of the pituitary adrenal axis with low doses of dexamethasone is associated with significantly greater levels of post-operative pain. Studies in animals were designed to

elucidate the role of pituitary B-END in modulating the perception of pain.

4-3. Animal Studies

4-3.a. The Role of Pituitary B-END in Mediating CRH-Induced Antinociception

The results of the rat hot plate studies indicate that secretion of pituitary iB-END is pivotally involved in the development of CRH induced antinociception. Preliminary studies demonstrated that a 25.2 nmole/kg dose of CRH reliably produced antinociception as compared to vehicle treated animals. Subsequent studies were directed towards testing the hypothesis that pituitary secretion of B-END mediates CRH antinociception.

One prediction of the B-END hypothesis is that the observed antinociception should be blocked by pretreatment with an opiate antagonist such as naltrexone. The results indicate that administration of naltrexone at both 1 mg/kg and 10 mg/kg completely blocked CRH antinociception, as compared to the CRH/vehicle treatment combination, at 15 minutes following injection. Blockade by the 1 mg/kg dose of naltrexone suggests the functional involvement of mu opiate receptors, since higher doses (e.g., 10 mg/kg) are generally considered necessary to block delta and kappa opiate receptors. The observation that CRH antinociception

is also blocked by a quaternary analog of naltrexone is consistent with the view that pituitary B-END acts on a opiate receptor in the periphery, outside the BBB.

The primary findings are that naltrexone blocks CRH antinociception. This blockade is not due to CRH binding to opiate receptors (Dave, et al. 1985b), nor is it due to naloxone blockade of B-END secretion (Fig. 23). Rather, we interpret the evidence to indicate that CRH stimulates the release of endogenous opioids. These findings are correctly predicted by the B-END hypothesis, but are not compatable with a direct action of CRH on the brain, or to an action due to release of other POMC derived peptides (e.g., ACTH).

A second prediction of the B-END hypothesis is that CRH antinociception should be dependent on an intact pituitary. In this experiment, hypophysectomy blocked the development of CRH antinociception, as compared to the sham surgery/CRH group. Although hypophysectomized rats had baseline nociceptive latencies identical to sham counterparts, the two groups behaved differently with repeated testing. The control group in this study (sham surgery/vehicle) demonstrated a decline in paw lick latencies observed over time, a finding generally observed in all studies. On the other hand, hypophysectomized rats treated with vehicle did not exhibit this decline in latency; their values remained near baseline throughout the testing session. A similar pattern was observed for the

hypophysectomy/CRH group. Although with repeated testing they had latencies greater than sham/vehicle, the levels were barely different from hypophysectomy/vehicle treated rats. In contrast, the sham/CRH group exhibited a significant level of antinociception throughout the experimental period as compared to sham/vehicle. Thus, hypophysectomy blocks CRH antinociception. These results are in accord with the pituitary B-END hypothesis and incompatable with a direct action of CRH or with a CNS release of endogenous opioids.

A third prediction of the B-END hypothesis is that CRH antinociception should be dependent on a functional pituitary corticotroph. The dose of dexamethasone employed in this study has been demonstrated previously to block corticotroph release of iB-END in response to a variety of stressful stimuli (Mueller, et al. 1985). This dose of dexamethasone completely suppressed corticotroph responsiveness to subsequent administration of CRH. In addition to blocking B-END secretion, dexamethasone blocked the development of CRH-induced antinociception. While glucocorticoid blockade of CRH antinociception is a testable extension of the pituitary B-END hypothesis, it is not predicted by other hypothesis such as a direct neural action of CRH or a CRH induced release of CNS opioids.

A fourth prediction of the pituitary B-END hypothesis is that the observed antinociception should be blocked by passive immunization with anti-B-END antisera.

It is important to note that the anti-B-END antisera does not bind to CRH, even at concentrations calculated to be three-fold in excess of peak blood levels of CRH. In addition to directly testing the possibility of analgesic actions of iB-END secreted from the pituitary, this study is the first to measure in vivo the total amount of iB-END secreted in response to CRH.

Preliminary studies demonstrated that there is a linear relationship between the amount of antisera present and the amount of iB-END bound. Thus, titer assays of blood from passively immunized rats were used to determine the remaining amount of free anti-endorphin antisera in units of femtomoles of iB-END binding/ml plasma. Passively immunized animals treated with CRH (vs. vehicle) have significantly increased rates of pituitary B-END release and thus, significantly lower levels of available iB-END binding. The difference in available binding between the CRH and vehicle groups is then a measure of the total amount of iB-END secreted by the pituitary corticotrophs due to CRH. This assumes no differences between the anti-endorphin/CRH and anti-endorphin/vehicle groups for degradation of antisera or antisera binding affinity. With these assumptions in mind, the pituitary corticotroph secretes approximately 3900 fm iB-END/ml plasma/75 minutes in response to CRH. In addition, the presence of substantial amounts of unbound antisera remaining in the anti-endorphin/CRH group implies that the actual amount of

free, or biologically active, iB-END is most likely very small.

The behavioral outcome of passively immunizing rats with anti-endorphin antisera is the blockade of CRH antinociception. The finding that passive immunization blocked CRH antinociception is compatible with the pituitary B-END hypothesis but is not predicted by other hypotheses such as a direct neural effect of CRH, release of other POMC derived peptides such as ACTH, or release of CNS opioids.

4-4. Review of Evidence in Support of the Pituitary B-END Hypothesis

4-4.a. Overview

The results of this research indicates that CRH antinociception is blocked by: 1) naltrexone; 2) hypophysectomy; 3) dexamethasone; and 4) anti-endorphin antisera. These findings strongly support the hypothesis that pituitary B-END release results in analgesia through an opioid mechanism, possibly in the periphery. Moreover, all of the above experiments constitute direct tests of this hypothesis. In addition, alterations in pituitary B-END levels are clinically relevant since CRH-induced increases in iB-END results in significant pain relief when administered to humans suffering from acute inflammatory

pain, while decreases in iB-END levels by a low dose of dexamethasone result in hyperalgesia.

4-4.b. Reconciliation With Pharmacological Studies

An obvious question arises from this conclusion. To produce analgesia, why are supraphysiologic doses of B-END necessary when it is peripherally administered? Several issues may relate to this apparent quandary. First, peripheral administration may not adequately mimic pituitary secretion of B-END. Pituitary secretory patterns, via retrograde flow, may place B-END directly into mediobasal hypothalamic regions; these areas are exquisitely sensitive to opiates for the production of analgesia (Tseng, et al. 1980, Satoh, et al. 1985, Kawajiri and Satoh 1985). In addition, stress is associated with increased BBB permeabilty (Hayes, et al. 1985), which may provide increased CNS access of pituitary B-END when secreted under conditions of stress.

A second issue relates to the pain model employed. Despite their popularity, the hot plate and tail flick methods are quite insensitive when compared to other animal models. Previous studies evaluating peripheral B-END used a 55 C hot plate or a tail flick test (Tseng, et al. 1976a). However, Ankier (1974) has demonstrated that the ED50 for morphine using the 55 C hot plate is three times greater than when tested on the 50 C hot plate. In other

words, when tested at 55 C, it takes three times as much morphine to produce the same level of antinociception as when tested at 50 C. Additionally, other methods of pain assessment, such as the acetic acid writhing test, have been estimated to be 10 times more sensitive for detecting antinociceptive effects of morphine as compared to the hot plate or tail flick test (Martin 1984).

Moreover, changes in the threshold for an acute noxious thermal stimulus (i.e., hot plate or tail flick) are probably not representative of all forms of pain. For example, non-steroidal anti-inflammatory drugs are potent and effective analgesics in models of inflammatory pain, but are not generally detected in either the hot plate or tail flick models. The prolonged biochemical changes subserving the development of hyperalgesia during the course of inflammation can be modulated by several types of endogenous factors - none of which may alter the ability of a thermoreceptor to detect an acute noxious thermal stimulus. In contrast to acute thermal stimuli, inflammatory pain may represent a more sensitive model to assess the analgesic activity of endogenous substances acting either centrally or peripherally.

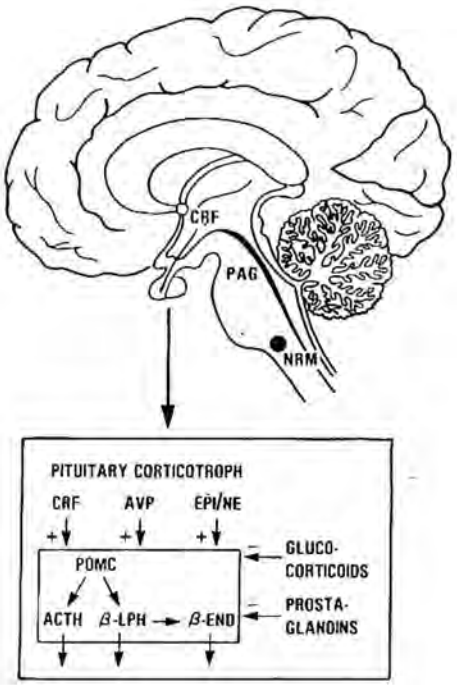
Taken together, these findings suggest that much larger doses of B-END may be required in pharmacological studies, where the route of administration does not mimic normal secretory patterns and the method of assessing analgesia is not sensitive.

The findings from this study, which directly tested the pituitary B-END hypothesis, are in agreement with the indirect evidence advanced by stress induced analgesia (SIA) studies. Indeed, most of the interventions employed in this project have been used to test for a pituitary B-END role in SIA. With the pivotal role of endogenous CRH in stimulating pituitary B-END secretion in response to stress, additional tests of B-END involvement in SIA could be made by utilizing a CRH antagonist (e.g., alpha CRH_{9-14}) or passive immunization with anti-CRH antisera. These forms of interventions would allow an accurate assessment of pituitary B-END involvement under various stress paradigms.

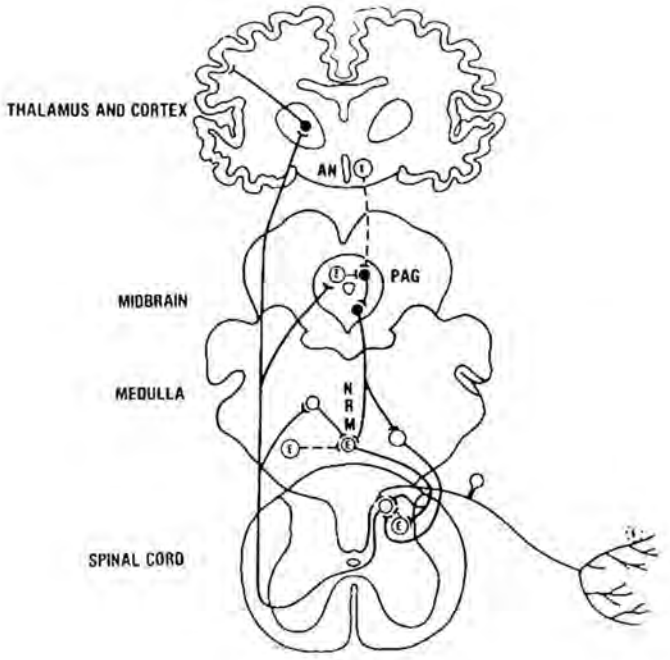
4-5. Implications of the Research

For purposes of summarizing the discussion on the role of B-END in attenuating nociception, a model is presented which schematically depicts the pituitary corticotroph and the potential target sites for pituitary B-END. As diagrammed in Figure 31, secretory activity of the pituitary corticotroph is modulated by several substances, with CRH and glucocorticoids comprising the major determinants of activity. Results from this study indicate that patients in acute pain have concurrent

Figure 31. Potential interactions between neuronal and humoral pain control systems. Left Panel: Regulators of pituitary corticotroph secretion of B-END. Right Panel: The endogenous neural pain control systems. The letter "E" symbolizes sites at which endogenous opioids can activate analgesic systems. See Figure 1 for details.



POTENTIAL INTERACTIONS BETWEEN NEURONAL AND HUMORAL PAIN CONTROL SYSTEMS



stimulation of B-END secretion and activation of an endogenous opioid analgesic system. There are several interactions that may occur between humoral and neuronal pain control systems. As previously discussed, pituitary B-END may modulate the development of pain at any of these neuronal areas. First, B-END may reach CNS regions, either by retrograde flow or by crossing the BBB. Likely target sites include the hypothalamus, periaquaductal grey and rostral medullary nuclei (Fig. 31).

A second potential target site is peripheral nociceptive nerve fibers possessing opiate receptors (Fig. 31). As reviewed in Chapter 1 (1-5.c), several lines of evidence, including both biochemical demonstration of opiate binding sites and pharmacological demonstration of peripheral opiate analgesia, support a peripheral site of opiate analgesia. In this study, the finding of blockade of CRH analgesia both by quaternary naltrexone and by peripheral passive immunization provides additional support for this hypothesis.

The results of these studies demonstrate that pituitary B-END is analgesic in humans and rats. Further, the findings suggest that blood-borne B-END produces analgesia through activation of opiate receptors located on peripheral nerves. The ability of circulating B-END to produce analgesia through actions on peripheral opiate receptors suggests new strategies for the treatment of clinical pain. Therapeutic approaches which modulate

pituitary B-END secretion or the function of peripheral opiate receptors may provide clinically effective analgesia without the side effect liability associated with central actions of narcotic analgesics.

APPENDIX

The following 8 pages include clinical summary sheets for the CRH study (page 1), the dexamethasone study (page 2), sample patient questionnaires used for both studies (pages 3-5) and a copy of the patient's informed consent document (pages 6-8).

CRH/ENDORPHIN STUDY

Pt. #_____ Name:_____ CC#_____ Date:_____

Sex:_____ Height:_____ Weight:_____ Age:_____

SUMMARY OF SURGERY:

Time of Local Anesthesia:_____

Amount of Local Anesthetic:_____

Time Surgery Started:_____

Time Surgery Completed:_____

(= zero minutes post-op)

Teeth Extracted: Surgical Trauma:

#1_____ _____

#16_____ _____

#17_____ _____

#32_____ _____

DRUG RESUSPENSION:

Resuspend vial (contains plbo or 85ug CRH) in 1.7ml sterile water (this gives a concentration of 50 ug/ml). Inject 0.2ml for every 10kg body weight to give a dose of 1.0 ug/kg.

VOLUME INJECTED:_____ml ---inject over 1 minute---

---inject at 60 min post-op---

SUMMARY OF DATA COLLECTION

Time Post-Op	= Time Post-Drug	Actual Time	QUESTIONS	BLOOD	EKG	BP	FLUSHING (+/-)	TEMP
Pre-op	-			*****	*****	****/****	********	*****
55'	= 5'pre					/		
75'	= 15'					/		
90'	= 30'					/		
105'	= 45'			*****		/		*****
120'	= 60'					/		
135'	= 75'			*****		/		*****
150'	= 90'					/		
180'	= 120'					/		

Plasma: Collect two 6ml purple tops and one 5ml green top vacutainer.

EKG: Run a strip for 30 seconds.

ENDORPHIN STUDY #2: I.V. POST-OP (DEX STUDY)

Pt# _____ Name: _____ CC# _____ Date: _____

Sex: _____ Height: _____ Weight: _____ Age: _____

SUMMARY OF SURGERY

TIME OF LOCAL ANESTHETIC: _____

AMOUNT OF LOCAL ANESTHETIC: _____

TIME SURGERY STARTED: _____

TIME SURGERY COMPLETED: _____

TEETH EXTRACTED	SURGICAL TRAUMA
#1 _____	_____
#16 _____	_____
#17 _____	_____
#32 _____	_____

SUMMARY OF DATA COLLECTION

Time Post-op	=	Time Post-Drug	Actual Time	QUESTIONS	BLOOD	
(Pre-op)	=	-	_____	_____	____	
(5')	=	-	_____	_____	____	
10'	=	0	_____	****GIVE DRUG****		****MOVE PATIENT TO RECOVERY ROOM****
30'	=	20'	_____	****ASSESS ANESTHESIA****		
40'	=	(30')	_____	_____	____	
70'	=	(60')	_____	_____	____	
100'	=	(90')	_____	_____	____	
130'	=	(120')	_____	_____	____	
160'	=	(150')	_____	_____	____	
190'	=	(180')	_____	_____	____	****REMOVE I.V.****
220'	=	(210')	_____	_____	*****	

CRH/ENDORPHIN STUDY

Pt. #_____ Observation_____

Date:_____ Time_____

1. HOW NERVOUS ARE YOU AT THIS TIME?

EXTREMELY_____

MODERATELY_____

SLIGHTLY_____

NOT AT ALL_____

2. PLACE A MARK ON THE LINE TO SHOW THE AMOUNT OF NERVOUSNESS YOU FEEL.

NOT NERVOUS ——— NERVOUS AS COULD BE

3. PLEASE TAP YOUR LOWER LIP AND DESCRIBE HOW IT FEELS:

NUMB_____

TINGLING_____

NORMAL_____

4. ON WHICH OF YOUR TWO APPOINTMENTS DO YOU FEEL THAT YOU HAD LESS POST-OPERATIVE PAIN?

THIS TIME_____

LAST TIME_____

NIDR PAIN MEASUREMENT QUESTIONNAIRE

Pt.#_____ Observation_____

Date_____ Time_____

PLEASE READ THE FOLLOWING:

There are two aspects of pain which we are interested in measuring: the intensity, how strong your pain feels, and the unpleasantness, how unpleasant or disturbing the pain is for you. The distinction between these two aspects of pain might be made clearer if you think of listening to a sound, such as a radio. As the volume of the sound increases, I can ask you how loud it sounds or unpleasant it is to hear it. The intensity of the pain is like loudness; the unpleasantness of pain depends not only on intensity but also on other factors which may affect you. There are scales for measuring each of these two aspects of pain. Although some pain sensations may be equally intense and unpleasant, we would like you to judge the two aspects independently. Please mark the line to indicate the relative intensity of your pain sensation; the further to the right, the greater the intensity. Similarly, mark the second line to indicate the relative unpleasantness of your pain sensation.

1. PLACE A MARK ON THE LINE TO SHOW THE INTENSITY OF THE PAIN THAT YOU FEEL:

NO SENSATION — THE MOST INTENSE SENSATION IMAGINABLE

|———————————————————————————————|

2. PLACE A MARK ON THE LINE TO SHOW THE UNPLEASANTNESS OF THE PAIN THAT YOU FEEL:

NOT BAD AT ALL — THE MOST INTENSE BAD FEELING POSSIBLE FOR ME

|———————————————————————————————|

MEDICAL RECORD	CONSENT TO PARTICIPATE IN A CLINICAL RESEARCH STUDY Adult Patient or Parent, for Minor Patient

INSTITUTE: National Institute of Dental Research

STUDY NUMBER: 083-D-174A PRINCIPAL INVESTIGATOR: Dr. Raymond Dionne

STUDY TITLE: Neuroendocrine Response to Surgical Stress and Postoperative Pain

INTRODUCTION

We invite you (or your child) to take part in a research study at the National Institutes of Health. It is important that you read and understand several general principles that apply to all who take part in our studies: (a) taking part in the study is entirely voluntary; (b) personal benefit may not result from taking part in the study, but knowledge may be gained that will benefit others; (c) you may withdraw from the study at any time without penalty or loss of any benefits to which you are otherwise entitled. The nature of the study, the risks, inconveniences, discomforts, and other pertinent information about the study are discussed below. You are urged to discuss any questions you have about this study with the staff members who explain it to you.

The National Institute of Dental Research is conducting a study on chemicals released by the body in response to the stress of surgery and post-operative pain: epinephrine, norepinephrine, cortisol and beta-endorphin. These chemicals are part of the body's response to stress and pain and are thought to be important in adapting to these problems. By studying their release under normal conditions, and when various drugs normally used in oral surgery are given, we hope to learn how the body responds to the stress and pain associated with minor surgical procedures such as oral surgery.

CONSENT FORM

I understand that as part of this study I will undergo an oral surgical procedure, the removal of third molars ("wisdom teeth"), under local anesthesia and, possibly, with intravenous premedication. I understand that no dental care other than the removal of third molars is offered. Local anesthesia will consist of mepivacaine (trade name Carbocaine) which is a drug routinely employed for this purpose. Possible, but rare, adverse reactions associated with local anesthesia include temporary facial paralysis, hematoma formation, inadvertant overdose and allergic reactions. I may serve in one of three possible studies. I understand that in one study there is a one in four chance (25%) that I will receive intravenous premedication consisting of either saline placebo, diazepam, naloxone or fentanyl. Saline placebo is inert and is included for comparison to the effects given to dental patients to promote relaxation and amnesia during the oral surgical procedure. I understand that the sedative effects of diazepam may last as long as 24 hours after it is given, and that I should not attempt to drive a car or operate any hazardous machinery for the 24 hours after surgery. I also understand that diazepam may cause venous irritation (thrombophlebitis) Fentanyl is a short acting narcotic analgesic similar to morphine which is also given routinely prior to oral surgery to reduce any pain not prevented by the local anesthetic. I understand that fentanyl depresses respiration slightly, but that this effect will not last

MEDICAL RECORD	CONTINUATION SHEET for either: NIH 2514-1, Consent To Participate In A Clinical Research Study NIH 2514-2, Minor Patient's Assent To Participate In A Clinical Research Study

STUDY NUMBER: 083-D-174A CONTINUATION: page 2 of 4 pages.

longer than one hour. Naloxone is a narcotic antagonist used clinically to treat overdoses of narcotic analgesics. I understand that when given alone it may cause irritability and, possibly, increase the pain of oral surgery not prevented by the local anesthesia. I have been asked to remain at the clinic for three hours following surgery and understand that I willI receive a standard postoperative pain medication before leaving the clinic, acetaminophen plus codeine.

I also am aware that I may serve as a subject on a second study in which I will receive local anesthesia and will remain at the clinic until the local anesthesia wears off. I understand that I will receive, intravenously, either saline placebo or dexamethasone. Dexamethasone (trade name Decadron) is an anti-inflammatory drug used by some oral surgeons to suppress the swelling associated with extractions. It may also have an effect on the release of beta-endorphin, one of the chemicals we are studying. I understand that this drug may suppress the release of cortisol from my adrenal glands but that levels return to normal within a week. I have been told that I will stay at the clinic an additional 2½ hours after surgery for further testing. Before leaving the clinic, I will be given a dose of the postoperative pain pills if needed and given a supply to take home.

I also am aware that I may be asked to serve as a subject on a third study involving 40 subjects out of the 168 total. This will require that I have my third molars removed at two appointments, approximately 2 weeks apart. On the first day, an upper and lower third molar will be removed. After surgery, I will receive either saline placebo or corticotropin releasing hormone (CRH) through an intravenous needle in my arm. On the second appointment, at least two weeks later, an upper and lower third molar will be removed and I will receive the alternative drug. Saline placebo is inert and is included for comparison to the effects of CRH.

CRH is the brain hormone that controls a part of the pituitary gland that responds to stress. The chemical structure of CRH has been discovered recently. It has been synthesized in the laboratory and is now available for testing in humans. We believe that giving a dose of CRH and measuring the response of the pituitary may provide information about how the body responds to stress. CRH is a naturally occuring hormone, but has only recently been given to humans. Studies here at the NIH, with 250 patients receiving the same dose as you will receive, have not shown any changes in blood pressure, pulse, temperature, respiration or other tests. 10% of these patients did report a temporary "flushing" (skin warmth). Nonetheless, it is possible that an unforseen reaction or side effect could occur when you are given CRH. For this reason you will be observed frequently during the test. Before you enter the study, you will receive a medical history, a physical examination and a routine blood sample to measure your blood count and to test your liver and kidneys. An electrocardiogram and urinalysis will also be done.

MEDICAL RECORD	CONTINUATION SHEET for either: NIH 2514-1, Consent To Participate In A Clinical Research Study NIH 2514-2, Minor Patient's Assent To Participate In A Clinical Research Study

STUDY NUMBER: 083-D-174A CONTINUATION: page 3 of 4 pages.

I understand that I will be receiving standard clinical care for my third molar removal. This may require a small surgical incision over the site of the third molar, the removal of bone with a drill, if needed, and the use of oral surgery instruments. Following the procedure, sutures may be used to close the incision and a gauze placed over the extraction site to help stop the bleeding. I am aware that the complications of third molar removal include delayed healing of the extraction site, commonly known as a "dry socket", (approximately 5% incidence), infection (approximately 5% incidence), perforation of the lining of the sinuses located in the upper jaw (less than 1% incidence), damage to the teeth adjacent to the third molars (less than 1%) and transient (approximately 1% incidence) or permanent (approximately 1 in 1000 incidence) altered sensation to my lower lip, chin or tongue. A rare complication of oral surgery is a fractured jaw. I also understand that possible adverse effects of the postoperative pain pills being used include drowsiness, dizziness, nausea, vomiting and allergic reactions. These problems are usually mild and transient.

I also agree to the following instructions:

1. I will not have anything to eat or drink after midnight of the day prior to surgery.
2. I will be picked up at the clinic and driven home by an adult.
3. I will not drive a car or operate hazardous machinery for 24 hours following surgery.

EDICAL RECORD	CONSENT TO PARTICIPATE IN A CLINICAL RESEARCH STUDY • Adult Patient or • Parent, for Minor Patient	Continuation: page 4 of 4 pages

STUDY NUMBER: 83-M-174A

OTHER PERTINENT INFORMATION

. Confidentiality. When results of a study such as this are reported in medical journals or at meetings, the identification of those taking part is withheld. Medical records of Clinical Center patients are maintained according to current legal requirements, and are made available for review, as required by the Food and Drug Administration or other authorized users, only under the guidelines established by the Federal Privacy Act.

'. Policy Regarding Research-Related Injuries. The Clinical Center will provide short-term medical care for any physical injury resulting from your participation in research here. Neither the Clinical Center nor the Federal government will provide long-term medical care or financial compensation for such injuries, except as may be provided through whatever remedies are normally available under law.

3. Payments. You will not be paid for taking part in this study. The Clinical Center does not charge for medications, doctors' care or hospitalization. Exceptions for Normal Volunteers are guided by Clinical Center and Normal Volunteer Office policies.

4. Problems or Questions. Should any problem or question arise with regard to this study, with regard to your rights as a participant in clinical research, or with regard to any research-related injury, you should contact the principal investigator, Dr. Raymond A. Dionne, or these other staff members also involved in this study, Dr. Donald Butler, Dr. Kenneth Hargreaves or Ms. Peggy Wirdzek;
Building 10, Room 3C311. Telephone: (301) 496-5483.
National Institutes of Health
Bethesda, Maryland 20205

5. Consent Document. It is suggested that you retain a copy of this document for your later reference and personal records.

COMPLETE APPROPRIATE ITEM BELOW, A or B:

A. Adult Patient's Consent.
I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby consent to take part in this study.

Signature of Adult Patient & Date Signed

B. Parent's Permission for Minor Patient.
I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby give permission for my child to take part in this study.
(Attach NIH 2514-2, Minor's Assent, if applicable.)

Signature of Parent(s) & Date Signed

(if other than parent, specify relationship

Signature of Investigator & Date Signed

Signature of Witness & Date Signed

PATIENT IDENTIFICATION	CONSENT TO PARTICIPATE IN A CLINICAL RESEARCH STUDY • Adult Patient or • Parent, for Minor Patient

NIH-2514-1 (6-80)

P.A.: 09-25-0099

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